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Research Article
Effect of Genetic Polymorphisms in *GH/Hpa*II and *MSTN/Dra*I Loci on Body Weight in Friesian Bull Calves

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Abstract

Objective: The *GH* and *MSTN* gene polymorphisms and their association with body weight were declared in a population of 100 Friesian bull calves. **Materials and Methods:** For DNA extraction, collection of blood samples was carried out from the studied animals. The PCR for *GH* and *MSTN* genes yielded fragments of 329 and 1346 bp, respectively. **Results:** The PCR-*Hpa*II digestion of 329 bp of *GH* gene revealed three genotypes: AA genotype possess undigested fragment (329 bp), AB genotype has three fragments (329, 224 and 105 bp) and BB genotype has two fragments (224 and 105 bp). The *GH* genotypes incidence and alleles frequency were calculated. For the 100 Friesian bull calves, genotypic frequencies for the AA, AB and BB genotypes were 0.1, 0.78 and 0.12, respectively and the allele frequencies for A and B allele frequencies were 0.49 and 0.51. Statistical analysis revealed that there was a significant effect of *GH* genotypes on body weight. The AB genotype possessed higher body weight than the other 2 genotypes. Regarding *MSTN* gene, PCR-*Dra*I digestion of 1346 bp fragment was monomorphic; where it yielded four fragments (505, 427, 321 and 93 bp) in all animals under study. **Conclusion:** The outcome of this study is that it highlights the effectiveness of *GH/Hpa*II locus as candidate marker for body weight in cattle rather than *MSTN/Dra*I.

Key words: Body weight, Friesian cattle, *GH* gene, *MSTN* gene, PCR-RFLP

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Competing Interest: The authors have declared that no competing interest exists.

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

INTRODUCTION

Growth traits of animals are of principle concern for breeder due to their determinant and pivotal economical value[1]. Development of molecular techniques makes scientists able to attain more precise and proficient selection objective by marker-assisted selection (MAS). The association of candidate gene polymorphism with growth traits uses MAS to predict such traits and makes it a candidate for use in breeding programs. It could also be used as an implement for early selection of high growth rate animals and to prevent economic losses that are afforded by wasteful breeding of undesirable animals[2]. Candidate gene discipline permits discovering variation among individuals in the population for definite region of the DNA and accelerates rate of response to selection for trait of interest (e.g., body weight)[3,4].

Bovine growth hormone consists of from 190-191 amino acids and has 22 kD molecular weight with 1800-bp length[5-7], 5 exons and 4 introns[8,9]. Using restriction fragment length polymorphism (RFLP) genetic marker, many variant regions have been stated in different sites of *bGH* gene[10]. The most important reported polymorphisms are mutations at the third intron (transition T to C) and the fifth exon (transversion C to G) which are identified by *Msp*I and *Alu*I restriction enzymes, respectively[11-13]. The *GH* has anabolic role, major regulator of postnatal growth and development, tissue growth as well as lipid, protein and carbohydrate metabolism[14]. The *GH* role has been detected in numerous tissues, including bone, adipose tissue and muscle, so *GH* can be used as a candidate gene for marker-assisted selection for growth traits in several livestock species, including cattle[15]. Many studies indicated the association between *GH/Hpa*II locus and growth traits[16-19]. However, most of that reported analysis revealed opposing results or fail to demonstrate relationship.

Myostatin (*MSTN*) is one of the transforming growth factor β (TGF-β) superfamily, which comprises proteins that are key events in cell growth and development. The *MSTN* represents a negative myogenesis regulator[20] by obstructing the *MyoD* and *Myo*5 factors, which are associated with the mechanisms of precursor cells differentiation into myoblasts[21]. The *MSTN* gene knockout is the principle cause of double muscling phenotype that refers to increase mass of skeletal muscle that occurs due to the muscle fibers hypertrophy or hyperplasia[22-25]. Bovine *MSTN* gene is located on chromosome 2 (BTA2) at 3.1 cM (centimorgan) from the centromeric region, next to microsatellite TGLA44[26]. It consists of 2 introns and 3 exons, with 1840 and 2033 nucleotides in the two intron and 373, 374 and 381 nucleotides in the three exons[27]. The mRNA codes for a protein with 375 amino acids[28]. *MSTN-Dra*I locus polymorphism relatedness with body weight is scare[29]. Therefore, the main objectives of this study were to investigate the effect of genetic polymorphisms in *GH/Hpa*II and *MSTN/Dra*I loci on Friesian bull calves body weight.

MATERIALS AND METHODS

Animals and experimental samples collection: In this study, 100 Friesian bull calves were used. The farm records revealed that; the weaning weight of the studied animals was 90 kg b.wt., the birth weight was 29-38 kg and the weaning age was 75-100 days. Disodium EDTA containing tubes were used to prevent coagulation of blood during collection of samples. Then, blood samples storage was carried out at -20°C till DNA extraction procedures. This study practice was established via the animal welfare and ethics committee and the faculty of veterinary medicine, Damanhour University.

DNA extraction: The genomic DNA extraction was done via DNA purification kit procedures (Jena Bioscience, Germany). On 1.5% agarose gel, DNA quality was evaluated then examined in the UV transilluminator and bands were visualized and photographed. Moreover, to get high yield, enough concentration and purity of DNA, quantification of the extracted DNA was done using using nanodrop (UV-Vis spectrophotometer Q5000/USA).

PCR amplification: Amplification of fragments of *GH* gene and *MSTN* gene was carried out with expected amplicon sizes of 329 and 1346 bp[7,29] respectively. The primer sequences are represented in Table 1.

The PCR was done in a reaction volume of 25 μL according with some modifications. The reaction consists of 2.5 μL of 10x Green buffer of Dream Taq (Thermo Scientific, Germany), 1 μL primer each primer forward and reverse (10 pmol), 0.5 μL of (10 mM) dNTP (Thermo Scientific, Germany), 0.5 μL Taq DNA polymerase (Thermo Scientific,

Table 1: Primer sequences and size of the amplified fragments

Genes	Sequence	Size (bp)	References
GH	F:5′-CCCACGGGCAAGAATGAGGC-3	329	Dybus[7]
	R:5′-TGAGGAACTGCAGGGGCCCA-3		
MSTN	F:5′-CCCTACAGAGGCCACTTCAA-3	1346	Zhang *et al.*[29]
	R:5′-CTCGCTGTTCTCATTCAGATC-3		

Germany) and 17.5 µL ddH_2O which finally added to 2 µL genomic DNA (20 ng $µL^{-1}$). The reactions were done in a thermal cycler (Sure cycler 8800, Malaysia) and thermal cycling program denaturizing at 94°C for 5 min, followed by 35 cycles at 94°C for 1min, annealing temperature 63°C for 1 min and extension at 72°C for 1 min, final step is the extension at 72°C for 10 min.

The PCR product of each sample (5 µL) and 100 bp DNA ladder (Thermo Scientific, Germany) were loaded in 2% (w/v) agarose gels in tris-borate-EDTA (TBE) buffer staining using ethidium bromide. The electrophoresis was carried out for 45 min at 100 V. The electrophoresis gel was examined on an UV transilluminator and bands were visualized and photographed.

Polymorphism detection: The PCR products of *GH* gene were cleaved by fast digest *Hpa*II (Thermo Scientific, #FD0514) (isomer of *Msp*I) at 37°C for 5 min; while, *MSTN* gene amplified fragments were digested with *Dra*I (Thermo Scientific, #ER0221) at 37°C overnight. The reaction volume was 30 µL consisted of 10 µL PCR product, 17 µL H_2o (dd water), 2 µL 10x buffer, 1 µL restriction enzyme. The polymorphism of the cleaved fragments recognition was carried out by agarose gel electrophoresis then their polymorphic pattern was obviously envisioned under U.V by gel documentation system.

Data analysis

Non-genetic factors adjustment: Based on established farm records, adjustment of the different recorded calf weaning body weight to 205 days of age body weight was done by deduction from birth weight, weaning weight and age using this equation[30]:

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

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

Association of *GH* and *MSTN* genotypes with body weight: Statistical analysis was accomplished 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 SAS[31] was used for to elucidate association between *GH* and *MSTN* genotypes and corrected body weight.

Gene and genotypic frequencies: Gene and genotypic frequencies were calculated based on the electrophoresis results, by allele counting[32] then chi-square was carried out for the observed counted genotypes for assessment Hardy-Weinberg equilibrium status and demonstration of genotype distribution in the studied population.

RESULTS

Specific primers were used to amplify precise DNA fragments 329 and 1346 bp of *GH* and *MSTN* genes, respectively. Restriction analysis of 329 bp PCR products of *GH* gene digested with *Hpa*II revealed three genotypes; AA genotype possess undigested fragment (329 bp), AB genotype has three fragments (329, 224 and 105 bp) and BB genotype has two fragments (224 and 105 bp) (Fig. 1).

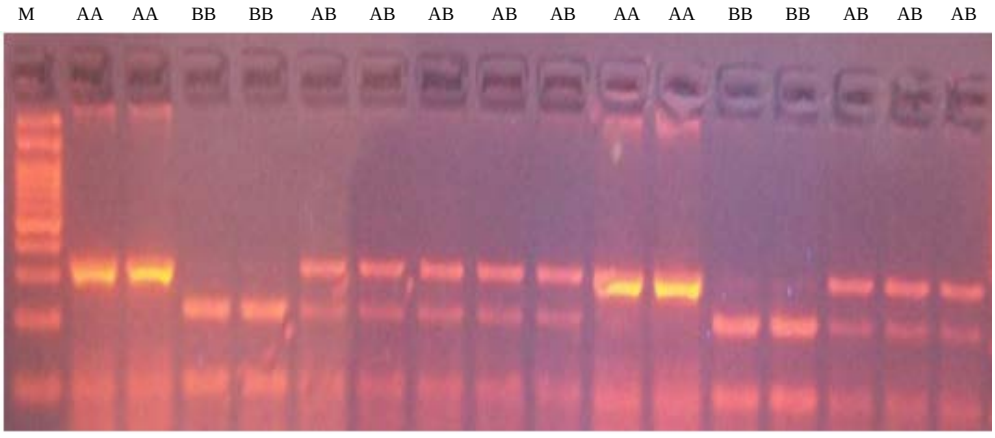

Fig. 1: Representative *Hpa*II restriction fragment pattern of *GH* gene (329 bp). AA: Restriction fragment of 329 bp, AB: Restriction fragment of 329, 224 and 105 bp, BB: Restriction fragment of 224 and 105 bp and M: DNA ladder 100 bp

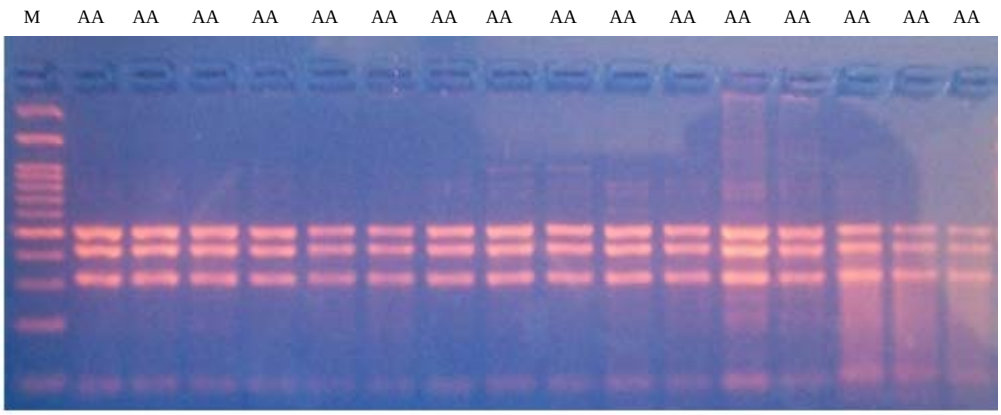

Fig. 2: Representative *Dra*I restriction fragment pattern of *MSTN* gene (1346 bp). AA: Restriction fragment of 505, 427, 321 and 93 bp, M: DNA ladder 100 bp

Table 2: Frequency of genotypes and alleles in the *GH* locus

	Genotyping frequency				Allele frequency	
Items	Total	AA	AB	BB	Allele A	Allele B
Observed	100	10/0.1	78/0.78	12/0.12	0.49	0.51
Expected		24.01	49.98	26.01		

Chi square calculated (χ^2) = 31.43 high significant differences, chi square tabulated (χ^2) at DF 1 and $p<0.05 = 3.84$

Table 3: Associations of *GH* genotypes with corrected body weight (Mean±SE)

Genotype	Means±SE of corrected body weight
AA	154.77±1.658[b]
AB	174.43±3.254[a]
BB	161.12±2.815[b]

Means of different levels within the same column having different superscripts are significantly different

Meanwhile, restriction analysis of 1346 bp PCR products of *MSTN* gene digested with *Dra*I was monomorphic; where it yielded four fragments (505, 427, 321 and 93 bp) in all animals under study (Fig. 2).

The *GH* genotypes incidence and alleles frequency were calculated. For the 100 Friesian bull calves, genotypic frequencies for the AA, AB and BB genotypes were 0.1, 0.78 and 0.12, respectively and the allele frequencies for A and B allele frequencies were 0.49 and 0.51. The χ^2-test exhibited that the GH genotypes distribution in the cattle population was not in Hardy-Weinberg equilibrium ($p<0.05$) (Table 2). Statistically significant *GH* genotypes effect on body weight was declared; where AB genotype possessed higher body weight trait than the other 2 genotypes. However, no significant variation was detected between AA and BB genotypes in the studied trait (body weight) (Table 3).

DISCUSSION

Growth rate represents economically important quantitative trait that affect carcass quantity in cattle. Genetic improvement of such trait has been accomplished using traditional methods of selection based on phenotypic information. However, candidate gene approach and molecular genetic markers could help to overcome some of the limitations of the traditional methods applied by animal breeder, genetic selection in this way has been very successful. The candidate gene approach refers to interpretation of polymorphisms of genes whose proteins are key enzymes involved vital physiological processes pathways[33]. When these genes are verified to be associated with particular economic trait, the existing traditional methods of trait selection have been substituted by the use of polymorphic specific genes that represent molecular detectable markers for economic traits of animals.

In this study, amplification of *GH* gene generated fragment length of 329 bp. The attained DNA cleaved fragments for *GH-Hpa*II digestion were: Digested (224 and 105 bp) fragments for BB genotype, three fragments (329, 224 and 105 bp) for AB genotype and undigested fragment (329 bp) for AA genotype. The frequencies of the detected various *GH-Msp*I genotypes in the 100 Friesian bull calves were 0.1, 0.78 and 0.12 for the AA, AB and BB genotypes respectively. The frequencies for A and B alleles were 0.49 and 0.51. The χ^2-test presented the obtained *GH* genotypes distribution in the cattle population not in Hardy-Weinberg equilibrium ($p<0.05$). Genotype counts for the observed and the expected values showed high significant difference indicating the population is not balanced and not follow Hardy- Weinberg equilibrium. This may be attributed to small sample size and/or artificial selection of parents for high body weight[34]. The *GH-Hpa*II locus was significantly associated with the desired trait of interest (body weight); where AB genotype was higher in body weight than the other 2 genotypes (AA and BB). Apposing results were

attained by previous studies to determine the association of *GH/Hapa*II variant and body weight in various cattle breed.

The *GH/Msp*I genetic diversity within and between 8 different breeds of beef cattle was reported[35-39]. On contrast, *Msp*I site in *GH* gene of Aceh cattle was monomorphic[40]. The frequency of AA genotype was 1.00 and same as A allele frequency.

Association between *GH/Msp*I locus genetic polymorphism and Grati dairy cow's body weight was studied[16]. The results showed that frequencies of A and B allele were 0.34 and 0.66, respectively. There was significant association between *GH/Msp*I genotypes and body weight ($p<0.05$). Also, *GH/Msp*I variant effect on body weight was identified in Ongole-crossbred cattle[17]. Results showed that cows and their subsequent progenies possessing the AB genotype performed the favorable body weight compared with BB and AA homozygous genotypes. Another study on *GH/Msp*I polymorphism and body weight, intron 3 of the *GH* gene polymorphism and body weight at first estrus and first calving in Holstein heifers in Antioquia was investigated[18]. Allele frequencies for the alleles (A) and (B) were 0.91 and 0.09, respectively. The genotype frequencies were 0.77, 0.2 and 0.03 for the genotypes (BB), (AB) and (BB), respectively. There was an association between genotype and weight at first estrus and first calving ($p<0.01$). On contrary, *GH/Msp*I locus did not significantly affect the body weight and measurements of West Sumatera Pesisir cattle[19].

In the present study, PCR-*Dra*I digestion of 1346 bp of *MSTN* gene was monomorphic; where it yielded four fragments (505, 427, 321 and 93 bp) in all animals under study; suggesting that *MSTN-Dra*I locus did not affect the body weight. *The MSTN-Dra*I locus polymorphism relatedness with body weight is scare. In three Chinese cattle breeds, PCR-*Dra*I digestion of 1346 bp of *MSTN* gene was polymorphic[29]. The following DNA fragments were attained according this previous study; fragment lengths of 505, 427, 321 and 93 bp for genotype AA, 505, 365, 321, 93 and 62 bp for genotype BB and 505, 427, 365, 321, 93 and 62 bp for genotype AB. According to results, *MSTN-Dra*I locus could affect body weight. Also, *MSTN* gene g-371 T>A promoter polymorphism and its association with carcass trait was screened in Holstein and Korean cattle breeds. Significant differences ($p<0.05$) were found between the *MSTN* genotypes and all carcass traits, except the live weight, back fat thickness, eye muscle area, carcass weight, marbling score, or meat color index[41] .

The limitation of the present study should be acknowledged. First, small sample size may not allow obtaining concrete conclusion. Second, limited number of candidate gene markers may influence the conclusion. Accordingly, such shortcoming should be considered in further investigations.

CONCLUSION

This pilot study highlights the significance effect of *GH/Hpa*II locus as candidate for body weight in cattle rather than *MSTN/Dra*I. Moreover, *GH/Hapa*II locus can be utilized as a marker for early selection of high body weight animals and early culling of lower body weight animal's results in preventing economic losses afforded by the latter. Further studies need to be done on a large sample size and wide range of cattle breed to establish the association between *GH/Hpa*II and *MSTN/Dra*I gene polymorphisms and body weight.

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