Kafkas Univ Vet Fak Derg
24 (6): 873-880, 2018
DOI: 10.9775/kvfd.2018.20193

Kafkas Universitesi Veteriner Fakultesi Dergisi
Journal Home-Page: http://vetdergi.kafkas.edu.tr
Online Submission: http://submit.vetdergikafkas.org

Research Article

Effects of *GH-Alu*I and *MYF5-Taq*I Polymorphisms on Weaning Weight and Body Measurements in Holstein Young Bulls [1]

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Article Code: KVFD-2018-20193 Received: 21.01.2018 Accepted: 23.08.2018 Published Online: 05.09.2018

How to Cite This Article

Çinar MU, Akyüz B, Metin Kıyıcı J, Arslan K, Kaliber M, Aksel EG: Effects of *GH-Alu*I and *MYF5-Taq*I polymorphisms on weaning weight and body measurements in Holstein young bulls. *Kafkas Univ Vet Fak Derg,* 24 (6): 873-880, 2018. DOI: 10.9775/kvfd.2018.20193

Abstract

Live weight and body measurements are economically important quantitative traits that affect carcass yield and calf survival in cattle. Four genes, growth hormone gene *(GH),* myogenic factor 5 *(MYF5),* fatty acid binding protein 4 *(FABP4)* and signal transducers and activators of transcription 5A *(STAT5A)* were chosen as candidate genes for live weight and body measurements due to their important role in growth. The aims of this study were to genotype *GH-Alu*I, *MYF5-Taq*I, *FABP4-Hin*II and *STAT5A-Ava*I polymorphisms and to investigate their associations with live weights and body measurements in Holstein young bulls. Genotyping of the single nucleotide polymorphism (SNP) markers in these candidate genes was carried out using the restriction fragment length polymorphism (RFLP) analysis. Frequencies of L allele for *GH,* A allele for *MYF5*, G allele for *FABP4* and C allele for *STAT5A* were, 0.96, 0.61, 0.79 and 0.74, respectively in the examined animals. The regression analysis indicated that the *GH-Alu*I polymorphism showed an association with weaning weight (WW), 180th day weight and hearth girth from birth to 180 days of age. The *MYF5-Taq*I polymorphism was found to influence body length at birth and birth weight (BW). However, no significant association was detected between the *FABP4-Hin*II genotypes and measured traits. The *GH-Alu*I and *MYF5-Taq*I polymorphisms may be useful for selection on live weight and body measurement traits in Holstein young bulls.

Keywords: *GH, Live weight, MYF5, RFLP, Weaning weight*

*GH-Alu*I ve *MYF5-Taq*I Polimorfizmlerinin Erkek Holstein Buzağılarında Sütten Kesim Ağırlığı ve Vücut Ölçüleri Üzerine Etkileri

Öz

Canlı ağırlık ve vücut ölçüleri gibi önemli kantitatif özellikler, karkas verimi ve buzağı yaşama gücünü etkilemektedirler. Büyümedeki rolleri nedeniyle büyüme hormonu geni *(GH)*, miyojenik faktör 5 *(MYF5)*, yağ asidi bağlayıcı protein 4 *(FABP4)* ve sinyal dönüştürücü ve transkripsiyonu aktive edici faktör 5A *(STAT5A)* canlı ağırlık ve vücut ölçüleri için aday gen olarak önerilmiştir. Bu çalışmada erkek Holstein buzağılarda *GH-Alu*I, *MYF5-Taq*I, *FABP4-Hin*II ve *STAT5A-Ava*I polimorfizmleri belirlenerek, elde edilen genotip verileriyle canlı ağırlık artışı ve vücut ölçüleri arasındaki ilişkilerin araştırılması amaçlanmıştır. İncelenen örneklerin bu aday genlerdeki SNP markırları yönünden genotipleri restriksiyon parçacık uzunluk polimorfizmi (RFLP) analiziyle belirlenmiştir. İncelenen örneklerde *GH* için L allel, *MYF5* için A allel, *FABP4* için G allel ve *STAT5A* için C allel frekansı diğer allelden yüksek bulunmuştur (sırasıyla 0.96, 0.61, 0.79 ve 0.74). Elde edilen genotip verileri kullanılarak yapılan regresyon analizi sonunda *GH-Alu*I polimorfizmi ile sütten kesimdeki (WW) ve 6. ay canlı ağırlıkları ile tüm ölçüm dönemlerindeki gögüs çevresi uzunluğu ile ilişkili olduğu görülmüştür. *MYF5-Taq*I polimorfizmi ise doğumdaki vücut uzunluğu ve doğum ağırlığı (BW) ile ilişkili bulunmuştur. İncelenen örneklerde *FABP4-Hin*II polimorfizmi ölçülen özelliklerin hiç biri ile ilişkili bulunmamıştır. Çalışma sonunda *GH-Alu*I ve *MYF5-Taq*I polimorfizmlerinin canlı ağırlık ve vücut ölçüleri yönünden yapılacak seleksiyon çalışmalarında kullanılabileceği düşünülmüştür.

Anahtar sözcükler: *GH, Canlı ağırlık, MYF5, RFLP, Sütten kesim ağırlığı*

INTRODUCTION

Beef is an excellent source of protein for human nutrition but, the production costs is higher compared to poultry and pork [1]. Therefore, increasing the meat yield is one of the most important issue in cattle breeding specially in countries in which their consumer preferences depending on mainly ruminant meat. Genomic selection

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is an encouraging development in livestock, proposing improved production by deciphering molecular genetic markers to design novel breeding programs and to develop new markers-based models for selecting favorable genotypes [2].

One of the best known genetic marker for beef yield is the growth hormone gene *(GH)* [3,4] which is encoding for growth hormone. *GH* is located on cattle chromosome 19 (BTA19) [5], and consists of five exons and four introns [6]. It is playing role in numerous physiological processes such as growth traits, mammary gland development and lactation [7]. Although, studies have mostly focused on association of *GH* genotype with milk yield traits, association of *GH* genotypes with live weight and body measurement traits have been less investigated [8]. Several polymorphisms were detected in the bovine *GH* gene, however, the best known of these polymorphisms is the leucine (L) to valine (V) substitution at position 127 in exon 5 of *GH* gene which creates a cut point for *Alu*I restriction enzyme [4].

Skeletal muscles occur result of a series of physiological processes which is named as myogenesis, and it includes cell specification, proliferation and differentiation, in which multi-potential mesodermal cells are differentiated into myoblasts [9]. Myogenesis begins at embryonic stage and continues to postnatal maturation [10] and controlled mainly by myogenic determination *(MyoD)* gene family [11]. Myogenic factor 5 *(MYF5)* gene is one of the four members of *MyoD* family [12] and located on BTA5 in bovine [13]. It is evaluated as a candidate gene for growth traits in beef cattle breeding because of its tasks in muscle development and growth traits [13,14].

The fatty acid binding protein 4 *(FABP4)* has got important tasks in lipid hydrolysis and intracellular fatty acid uptake in different tissues [15,16]. This protein is encoded by *FABP4* gene and its mRNA expresses firstly in adipocytes [17] from a locus located on BTA14 in cattle [18]. In literature, few reports were shown the relationship between *FABP4* and carcass weight in native Korean cattle breed [19,20]. However, no study was devoted to association *FABP4* variants with live weight and body measurements in Holstein cattle.

Signal transducers and activators of transcription protein (STAT) is a family of transcription factors which is mediated the effects of some peptide hormones and cytokines. STAT family has seven members [21], of them *STAT5A* is an important mediator of growth hormone which is located on BTA19 in bovine [22]. Therefore, it was thought that *STAT5A* gene may be used as a marker for important yield traits such as growth and live weight gain in livestock [23].

Although functional relation of these genes in growth metabolism has been reviewed in literature, association of polymorphisms in these genes with live weight traits and body measurements have not been strongly taken into consideration in Holstein cattle breed. Therefore, aims of this study were to investigate SNPs in *GH*, *MYF5*, *FABP4* and *STAT5A* genes, and investigate their association with live weight traits and some body measurements in Holstein young bulls reared in Turkey.

MATERIAL and METHODS

The project was approved by the relevant Animal Ethics committee of Erciyes University (#13/72 10.04.2013). A total of 59 male Holstein calves, born between March 2013 and November 2014 were used in this study. Animals were not applied to a special feeding program. Calves were stay with their mother after birth and consume *ad-libitum* colostrum for three days. At the end of three days, calves were fed with milk 10% of their birth weight until weaning. At seven days old, calf starter feed which contains calve growing feed and forages were provided ad-libitum until weaning. After weaning, calves were fed with milk replacer for 40 days until milk replacer weaning day. Young bulls were fed with forage and beef cattle feed (concentrated protein) mixture until 180th day. As live weight traits; birth weight (BW), weaning weight (30. day) (WW), milk replacer weaning weight (70. day) (RW) and weight at 180th day after birth (SW) were measured. The animals were weighed to the nearest kilogram using an electronic weighing scale (EziWeigh 5i, Tru-Test, New Zealand) mounted on a concrete platform. For body measurements; body length, wither height and hearth girth were measured. Body measurements were taken by two observers using an ordinary measuring tape and recorded in centimeters. Body length was measured as the distance from *Atriculus huneri* to *Tuber ichii*; wither height was measured as the distance from the ground to the highest point of wither. Hearth girth was measured as behind the front shoulder at the fourth ribs, posterior to the front leg [24].

All blood samples were collected post-natal period, and genomic DNA was extracted by the phenol:chloroform:isoamlyalcohol method. The *GH*, *MYF5*, *FABP4* and *STAT5A* gene polymorphisms were genotyped by using PCR-RFLP. The PCR reactions mixtures of all genes were prepared as total volume of 25 µL, including 1.5 mM $MgCl_2$, 200 µM dNTP, 5 pmol of forward and reverse primer of each gene, 1×PCR buffer, 1U Taq polymerase and approximately 100 ng DNA. The PCR protocols for investigated genes were as follow: for *GH* gene, 4 min at 94°C for, then 40 cycles of 94°C for 40 s, 60°C for 40 s, 72°C for 40 s and the final extension at 72°C 10 min; for *MYF5* gene, initial denaturation at 94°C for 4 min, then 38 cycles of 94°C for 1 min, 64°C for 30 s, 72°C for 1 min and final extension at 72°C for 4 min; for *FABP4* gene, initial denaturation at 95°C for 4 min, then 32 cycles of 94°C for 1 min, 62°C for 1 min, 72°C for 1 min and final extension at 72°C for 10 min; for *STAT5A* gene, pre-denaturation at 95°C for 4 min, then 34 cycles of 94°C for 1 min, 64°C for 1 min, 72°C for 1 min and final extension at 72°C for 10 min. The obtained PCR products were digested by 5 U of restriction endonuclease enzymes according to

their own protocols (Fermentas, Thermo Fisher Scientific Inc., Waltham, MA, USA). After digestion procedures, the genotypes were detected on 3% (for *GH* and *STAT5A* genes) and 2% (for *FABP4* and *MYF5* genes) agarose (Prona Agarose; Basica Le, Burgos, Spain) gel electrophoresis. The nucleotide sequences of PCR primers and restriction enzymes used for genotyped amplification and RFLP process are presented in *Table 1*.

Allele and genotype frequencies of genes, investigated in the present study were calculated by using OEGE - Online Encyclopedia for Genetic Epidemiology studies online tool [29]. Statistical analyses were performed by using IBM SPSS Statistics 22.0 software. For *GH*, *MYF5*, *FABP4* and *STAT5A* genes genotype and phenotype associations were investigated by using independent general linear model (GLM) procedure and Duncan test for significance levels; $P<0.1$ and $P<0.05$. Sire (n=11) were assumed random factor in the GLM model. The statistical model used as follows:

$$Y_{ij} = \mu + S_j + G_i + e_{ij}$$

Where Y_{ij} is the observation of the weaning weight and body measurements traits; μ is the overall mean for each trait, S_j is the random effect of j^{th} sire, G_i is the fixed effect of i^{th} genotype for the relevant polymorphism and e_{ij} is the random residual error.

RESULTS

The 223 bp products were obtained after PCR process and PCR products were digested by *Alu*I restriction enzyme for *GH* gene. At the end of digestion, it was expected one band (223 bp) for VV genotype, three bands (223, 171 and 52 bp) for LV genotype, two bands (171 and 52 bp) for LL genotype. The band of 52 bp could not be observed on agarose gel electrophoresis. However, two bands (223 and 171 bp) were enough for genotyping *(Fig. 1a)*. The LL genotype was found to be the highest frequency, and the VV genotype was found to be the lowest frequency in our investigated Holstein population. The L allele frequency was higher than V allele *(Table 2)*.

The 490 bp products were obtained after PCR process and they were digested by *Taq*I restriction enzyme for *MYF5* gene. At the end of digestion, it was observed one band (490 bp) for AA genotype, three bands (490, 367 and 123

Table 1. *Primer sequence, accession number, PCR product size*

Gene	Accession Number	Sequence	Product Size	Restriction Enzyme	Reference
GH	EF592534.1	GCTGCTCCTGAGGGCCCTTCG GCGGCGGCACTTCATGACCCT	223 bp	*Alu*I	[25]
MYF5	M95684.1	AGAGCAGCAGTTTTGACAGC GCAATCCAAGCTGGATAAGG	512 bp	*Taq*I	[26]
FABP4	NC007312	ATTATCCCCACAGAGCATCG ACAAGACTTGGCCTCAAGGA	399 bp	*Hin*II	[27]
STAT5A	AJ237937	CTGCAGGGCTGTTCTGAGAG TGGTACCAGGACTGTAGCACAT	215 bp	*Ava*I	[28]

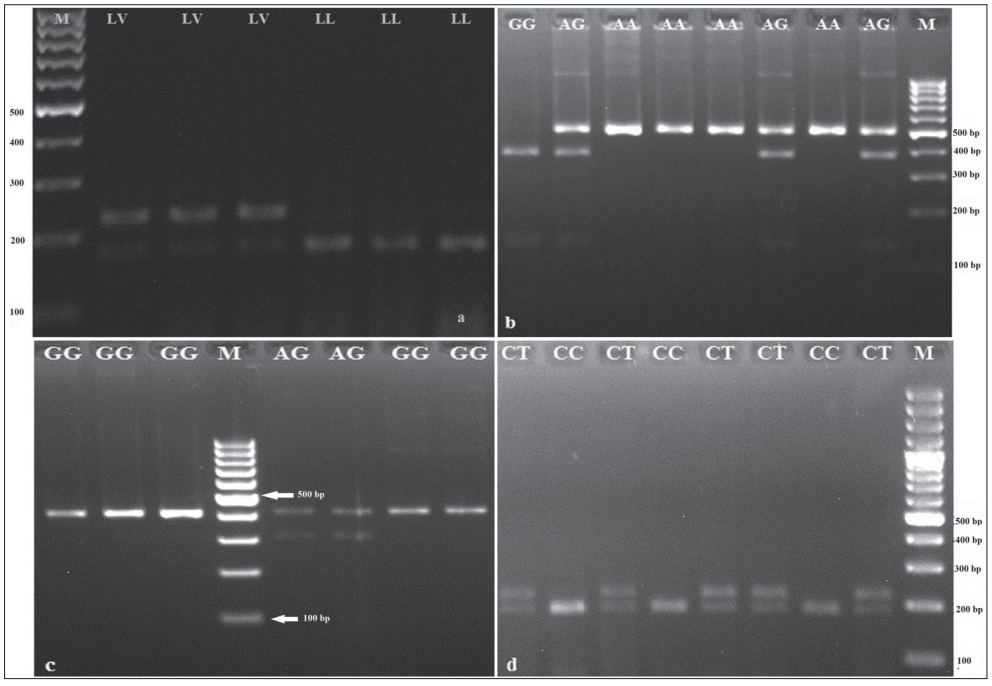

Fig 1. Agarose electrophoresis pattern of GH (a), MYF5 (b), FABP4 (c) and STAT5A (d). M: molecular marker (100 bp)

Table 2. *Genotype and allele frequencies of the GH, MYF5, FABP4 and STAT5A genes in beef Holstein cattle*

Gene	Genotype						Allele Frequency		Chi-squared
GH	LL		LV		VV		L	V	χ^2=0.12 P=0.734 (df=1)
	Obs (Exp)	F	Obs (Exp)	F	Obs (Exp)	F	0.96	0.04	
	54 (54.11)	0.915	5 (4.79)	0.085	0 (0.11)	-			
MYF5	AA		AG		GG		A	G	χ^2=1.16 P=0.2819 (df=1)
	Obs (Exp)	F	Obs (Exp)	F	Obs (Exp)	F	0.61	0.39	
	20 (21.97)	0.339	32 (28.07)	0.542	7 (8.97)	0.119			
FABP4	AA		AG		GG		A	G	χ^2=4.26 P=0.0389 (df=1)
	Obs (Exp)	F	Obs (Exp)	F	Obs (Exp)	F	0.21	0.79	
	0 (2.65)	-	25 (19.7)	0.424	34 (36.25)	0.576			
STAT5A	CC		CT		TT		C	T	χ^2=7.49** P=0.0062 (df=1)
	Obs (Exp)	F	Obs (Exp)	F	Obs (Exp)	F	0.74	0.26	
	28 (32.07)	0.475	31 (22.86)	0.525	0 (4.07)	-			

Obs: Observed genotype; Exp: Expected genotype; F: Frequency; df: degree of freedom

Table 3. *Least squares means and standard errors for BW, WW, RW and SW in male calves according to GH, MYF5, FABP4 and STAT5A genotypes*

Gene	Genotype	N	Traits			
			BW (kg) Mean (±SE)	WW (kg) Mean (±SE)	RW (kg) Mean (±SE)	SW (kg) Mean (±SE)
GH	LL	54	42.38±1.13	53.37±1.25[a]	73.21±1.71	188.57±4.78
	LV	5	39.80±2.70	46.89±2.98[b]	72.43±4.09	166.30±11.41
P			0.360	0.041	0.856	0.065
MYF5	AA	20	45.13±6.57	55.61±6.47	72.88±8.21	185.05±24.63
	AG	32	41.88±4.17	53.55±6.13	76.71±8.87	180.22±19.07
	GG	7	40.71±2.30	54.20±4.54	76.34±5.72	185.14±28.44
P			0.078	0.719	0.303	0.930
FABP4	AG	25	42.47±1.31	52.72±1.51	72.87±1.98	185.25±5.71
	GG	34	41.69±1.33	52.54±1.53	73.38±2.00	186.83±5.78
P			0.603	0.918	0.819	0.807
STAT5A	CC	28	42.23±1.33	52.68±1.53	75.59±1.90[a]	184.38±5.78
	CT	31	41.95±1.34	52.59±1.54	70.61±1.91[b]	187.69±5.81
P			0.858	0.959	0.028	0.622

Statistical differences among genotypes were shown as: [a] and [b] P<0.05

bp) for AG genotype, two bands (367 and 123 bp) for GG genotype *(Fig. 1b)*. The AG genotype was found to be the highest frequency whereas GG genotype was found to be the lowest frequency in our animals. The A allele frequency was found higher than G allele *(Table 2)*.

The 399 bp products were obtained after PCR process and they were digested by *Hin*II restriction enzyme for *FABP4* gene. At the end of digestion, it was observed one band (399 bp) for GG genotype, three bands (399, 302 and 97 bp) for AG genotype, two bands (302 and 97 bp) for AA genotype *(Fig. 1c)*. The GG genotype was found to be the highest frequency; however, the AA genotype was not found. The G allele frequency was higher than A allele *(Table 2)*.

The 215 bp products were obtained after PCR process and PCR products were digested by *Ava*I restriction enzyme for *STAT5A* gene. At the end of digestion, it was observed one band (215 bp) for TT genotype, three bands (215, 181 and 34 bp) for CT genotype, two bands (181 and 34 bp) for CC genotype *(Fig. 1d)*. The CT genotype was found to be the highest frequency, and the TT genotype was not detected. The C allele frequency was higher than T allele *(Table 2)*.

Association was found between *GH* and WW. The calf with *GH*-LL genotype had higher WW than other genotype (P<0.05) *(Table 3)*. *STAT5A* was found associated with RW. For *STAT5A* genotype CC had highest RW compared to other genotype CT (P<0.05) *(Table 3)*. Additionally, prospective associations were identified between *GH*-LL and higher SW

Table 4. *Least squares means and standard errors for body lengths in calves according to GH, MYF5, FABP4 and STAT5A genotypes*

Gene	Genotype	N	Traits			
			Body Length at Birth (cm) Mean (±SE)	Body Length at Weaning Old (cm) Mean (±SE)	Body Length at Milk Replacer Feed Old (cm) Mean (±SE)	Body Length at 180th Day (cm) Mean (±SE)
GH	LL	54	67.39±0.797	74.35±0.68	78.91±0.67	106.74±1.46
	LV	5	69.43±1.903	71.76±1.62	76.56±1.60	99.90±3.48
P			0.306	0.128	0.161	0.063
MYF5	AA	20	68.70±4.13[a]	74.15±4.11	78.35±3.36	106.65±6.72
	AG	32	67.03±2.82[ab]	74.31±2.40	78.59±3.36	106.34±6.37
	GG	7	65.29±3.45[b]	74.86±3.08	79.57±3.36	108.86±6.41
P			0.035	0.744	0.623	0.531
FABP4	AG	25	67.37±0.93	74.11±0.80	78.44±0.79	106.14±1.74
	GG	34	67.89±0.94	74.01±0.81	78.86±0.80	105.77±1.77
P			0.620	0.914	0.641	0.850
STAT5A	CC	28	67.98±0.94	73.97±0.81	78.62±0.80	104.28±1.72
	CT	31	67.25±0.94	74.15±0.82	78.66±0.80	107.66±1.73
P			0.505	0.850	0.966	0.095

Statistical differences among genotypes were shown as: [a] *and* [b] *P<0.05*

Table 5. *Least squares means and standard errors for HG in calves according to GH, MYF5, FABP4 and STAT5A genotypes*

Gene	Genotype	N	Traits			
			HG at Birth (cm) Mean (±SE)	HG at Weaning Old (cm) Mean (±SE)	HG at Milk Replacer Feed Old (cm) Mean (±SE)	HG at 180th Day (cm) Mean (±SE)
GH	LL	54	80.56±0.71	87.22±1.04	92.01±1.11	131.81±1.29
	LV	5	77.43±1.70	82.38±2.48	86.53±2.64	125.98±3.07
P			0.081	0.065	0.050	0.072
MYF5	AA	20	81.70±4.40	88.60±4.74	92.30±4.54	131.65±8.280
	AG	32	80.63±2.85	87.00±4.72	90.78±5.01	130.84±5.036
	GG	7	80.14±2.73	88.00±5.57	93.43±6.99	131.86±7.105
P			0.541	0.613	0.483	0.935
FABP4	AG	25	80.74±0.84	86.82±1.24	91.53±1.33	131.16±1.54
	GG	34	79.65±0.85	86.51±1.26	91.24±1.35	131.13±1.55
P			0.253	0.826	0.847	0.988
STAT5A	CC	28	80.12±0.86	85.77±1.24	90.32±1.32	130.39±1.55
	CT	31	80.29±0.86	87.57±1.24	92.47±1.33	131.91±1.55
P			0.863	0.213	0.165	0.396

(P=0.065) and *MYF5*-AA and higher BW (P=0.078) *(Table 3)*. Calves with *MYF5*-AA genotype had highest body length at birth than other genotypes (P<0.05) *(Table 4)*. Additionally, prospective association was identified between *GH*-LL (P=0.063) and *STAT5A*-CT (P=0.095) and longer body length at 180th day *(Table 4)*. Prospective association was identified between *GH* genotypes and hearth girth (HG) at different ages *(Table 5)*. *GH*-LL genotype was found associated with longer HG in all measured ages *(Table 5)*. No association was found between *FABP4* genotypes and investigated traits in this study.

DISCUSSION

To the best of our knowledge there is only few data about association between *GH*, *MYF5*, *FABP4*, and *STAT5A* genes polymorphisms with live weight and morphological measurements in Holstein cattle breed. In the present study genotype frequency of *GH*-LL was found most abundant compared to other genotypes *(Table 2)*. Similar results were observed in other Holstein populations from different countries. Frequency of genotype VV was found low or none in different Holstein populations [30-33].

These findings are consistent with our results, obtained in this study. Among investigated genes in our study, most promising association results were obtained from *GH* gene regarding live weight and body measurement traits in Holstein young bulls *(Table 3, 4* and *5)*. *GH*-LL genotype was associated with higher weaning weight compared to *GH*-LV *(Table 3)*. To the best of our knowledge, no association study was found regarding *GH-Alu*I polymorphism and WW in Holstein cattle breed. However, association between *GH-Alu*I polymorphism and weaning weight investigated in Canchim cattle [34] and Charolais and Zebu hybrid together with Charolais, Nelore and Canchim hybrid [35] and in all investigated genotypes no association was observed between *GH-Alu*I polymorphism and WW. Additionally, *GH-Alu*I polymorphism was found prospectively associated with SW *(Table 3)* and 180th day body length *(Table 4)*. In literature it was stated that *GH-Alu*I genotypes effects the growth and feed intake levels in cattle [36,37]. Additionally, it was shown that GH concentration is affected from *GH-Alu*I genotypes and age in cattle [36]. In the present study *GH-Alu*I genotypes found only associated with WW, SW and body length at 180th day but not with the other traits *(Table 3* and *4)*. We thought that this may be due to effects of environmental factors such as feed intake on *GH* genotypes. According to our literature search no study was found investigating effects of *GH-Alu*I genotypes with heart girth measurement. Heinrichs et al.[38] reported estimation of dairy heifer body weight from heart-girth measurements by using equations or tables. In the present study, heart girth was found prospectively associated with *GH-Alu*I genotypes in all periods *(Table 5)*, therefore it is thought that *GH-Alu*I polymorphisms might be used in selecting cattle with longer heart girth which may cause higher body weights.

In the current study all three genotypes for *MYF5-Taq*I polymorphism were observed in the investigated Holstein population *(Table 2)*. *MYF5-Taq*I AG genotype frequency was found most abundant compared to other two genotypes *(Table 2)*. Similar results were observed in different cattle breeds in Korean [39] and Turkish native cattle breeds [26] that genotype AG was found more abundant than two other genotype. There are not so many researches about association of *MYF5* genotypes with body weight in Holstein cattle. Nasr et al.[40] found an association between body weight and another *MYF5-Taq*I site. However, in the current study we only found association with birth weight in young Holstein bulls. As a member of myogenic regulatory factors, *MYF5* is an important transcription factor for skeletal myogenesis in mammalian embryos [41]. Because of its crucial role in embryonic growth, we thought that *MYF5* variants may affect birth weight and body measurements at birth. Supporting this idea above, in the present study, *MYF5-Taq*I polymorphism was found associated with body length at birth *(Table 4)* and prospectively associated with BW *(Table 3)*. Similarly, an association between *MYF5-Taq*I polymorphism and birth weight was reported in a Canadian commercial beef cattle population developed from various cattle breeds [13]. However, no association found in terms of same polymorphism in Korean and Chinese native cattle populations [39,42]. Our anticipation on inconsistent association results may resulted from breed differences. Because distinct genetic difference between European and Asian cattle breeds has been shown in literature [43].

In our study *MYF5-Taq*I GG genotype calves had lower birth weight and body length compared to other genotypes, however, animals those have low birth weight and body length are compensating these traits and reaching to similar body weight and length of animals with AA and AG genotypes. Probability of perinatal mortality was found higher in calves with heavier birth weight and birth weights above 42 kg shown as at high risk of perinatal mortality [44]. Therefore, selecting animals with GG genotype may lead us to select calves with lower risk of perinatal mortality without any economic loss at the slaughter age.

For *FABP4-Hin*II polymorphism genotype GG was the most abundant and genotype AA was none in the Holstein population *(Table 2)*. Similarly, in the Korean Hanwoo cattle genotype GG was also found as common genotype and frequency of AA was found as lowest (0.07) [18]. And no association was observed between *FABP4-Hin*II polymorphism and investigated phenotypes in Holstein young bulls.

In the present study, no *STAT5A-Ava*I TT genotype was observed in Holstein male calves. Similarly, this genotype was not found also in Polish Black-and-White [23] and Holstein cattle breeds [37]. However, TT genotype was observed in Podolica bulls [28] and Polish native cattle [23]. *STAT5A-Ava*I polymorphism was found associated with RW in our investigated animals *(Table 3)*, CC genotype had shown higher RW compared to CT genotype. In consistent with our results, *STAT5A-Ava*I CC genotype was found favorable for body weight in different ages from different cattle breeds [23,28,37].

Taken together, results in our study provide evidence that interaction between *GH-Alu*I and *MYF5-Taq*I polymorphisms have potential effects for growth and morphological traits which are correlated with economical traits in Holstein cattle. Further studies are ultimately needed to use the SNPs of these two candidate genes in larger populations for genomic selection and investigate other polymorphisms those are linked with *GH-Alu*I and *MYF5-Taq*I SNPs for growth related traits in cattle.

Conflicts of Interest

The authors declare no conflict of interest

Acknowledgments

This work was supported by Erciyes University, Scientific Research Project Coordination Unit (Grant number FCD-

2013-4640). Authors are indebted to Saray Çiftliği-Kayseri farm management for providing animals and helps during collection of phenotypes.

Authors' Contributions

MUC: Made experimental design and wrote the manuscript. JMK: Collected phenotype and samples and performed statistical analysis. KA: Performed laboratory experiments, responsible for chemical and reagents. EGA: Performed laboratory experiments. MK: Collected phenotype and samples. BA: Made experimental design and wrote the manuscript

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