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Determination of the *Alu*I polymorphism effect of bovine growth hormone gene on carcass traits in Zavot cattle with analysis of covariance

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Abstract: The aim of this study was to determine the genotypes of bovine growth hormone and to investigate their association with daily weight gain in the Zavot cattle breed. The bovine growth hormone (GH) genotypes of 45 male Zavot cattle were identified by PCR-RFLP. The effect of the bovine growth hormone gene on daily weight gain was estimated from a dataset obtained from cattle up to the age of 9 months. The mean differences of carcass weight or carcass yield among the genotype groups were assessed by analysis of covariance (ANCOVA). Two alleles for bovine growth hormone locus, L and V, were observed in Zavot cattle. The frequency of the L and V alleles was found to be 0.767 and 0.233, respectively. The genotype effect on live weight was not found to be significant ($P > 0.05$). The difference between the means of genotype groups was not found to be significant for carcass weight and carcass yield ($P > 0.05$). Further research on the association between bovine growth hormone gene polymorphism and yield traits in different cattle breeds should be conducted.

Key words: Carcass traits, covariance, growth hormone gene, polymorphism, Zavot cattle

1. Introduction

Economically important yield traits in farm animals largely consist of complex and continuously distributed phenotypes, which are influenced by multiple polygenes located at quantitative trait loci (1). The prediction of the future performance of selected animals is perhaps one of the most complex tasks in animal breeding (2). Nowadays, there is great international interest in furthering knowledge about the molecular structure of complex quantitative traits, and in directly establishing the genetic merit of the individual (3). For this purpose, candidate genes can be used in livestock. Candidate genes can be defined as genes with biological effects on a trait of interest, or as genes closely linked to affect the yield of a particular gene. Polymorphisms within a candidate gene can be detected because of their association with yield traits, and can also be used in the development of a marker-assisted selection (MAS) program. The combined use of MAS and conventional selection methods may be most effective for complex traits, such as shortening the generation interval and accelerating genetic improvement (4). However, there is not as yet sufficient information about the effect of the many variants of the candidate genes and possible genetic markers on livestock (1).

In recent years, significant developments in molecular genetic techniques have been used as an additional tool in conventional livestock breeding and selection strategies (5–7). As a result, genes or markers that may affect the economically important traits of livestock have been identified. Nowadays, there is increasing interest in using a positional candidate gene that affects economically important traits in livestock species, such as fertility, milk, and meat yields (2,8). Several polymorphisms in various genes have been reported to affect production traits in different livestock (8). The detection of sequence variations of genes affecting the yield traits in farm animals is now possible (7). More than 3600 marker loci have been mapped in the bovine genome and are extensively used in the search for genes with significant effects on quantitative traits. Until recently, the direct selection of candidates for the breeding of specific alleles was limited, mainly because of the lengthy and extensive progeny testing procedures required. Nowadays, however, molecular genetic techniques have become available, allowing direct genotyping for candidate genes in livestock farming (9).

Growth, weight gain, and carcass composition are economically important yield traits in livestock, and these traits are controlled by multiple genes. The selection

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of animals with higher growth rate and better carcass composition is of great significance to breeders for the improvement of native breeds. Current molecular genetic technologies could help scientists improve the accuracy and efficiency of traditional selection methods by applying genetic markers. Therefore, the determination of genetic polymorphisms in potential marker loci that are significantly associated with certain traits of interest is very useful in farm animal breeding (10). Recently, researchers and breeders have begun to select animals that are important for milk and meat production by means of several marker genes, such as κ-casein, β-1actoglobulin, GH, and prolactin (PRLL) (11).

GH directly or indirectly plays a notable role in tissue growth and fat metabolism. Thus, it has an important role in reproduction, lactation, and growth stimulation in animals (9,12). Consequently, there is great interest in using the GH gene to improve production traits in different cattle breeds (6). In addition, investigations of this gene may be important for improving productivity in both dairy and beef cattle breeding. Because of these important associations, the GH gene is thought to be a possible marker gene for marker-assisted selection programs in cattle (13).

The GH gene is located in bovine chromosome 19 (12). In addition, researchers have identified several polymorphisms in the GH gene (14). The best-known of these polymorphisms is the leucine (L) to valine (V) substitution at position 127 in exon 5 in the GH gene, which can be characterized using the *Alu*I restriction enzyme (1). Nowadays, the association between production traits (milk production, milk quality, growth, carcass composition, and carcass quality) and the effects of GH gene polymorphisms are being studied in cattle (15).

Zavot cattle are a native Turkish cattle breed found in the Kars and Ardahan provinces of northern Turkey. This breed is the least recognized native Turkish cattle breed and is under threat of extinction. The breed was established approximately 150 years ago as a result of crossbreeding between Simmental, Brown Swiss, and native East Anatolian Red cattle breeds (16). The Zavot cattle breed has been accepted as a combined productive breed. The colors of this breed are generally white with a white tint, light skin, and white hair. However, dark- or brown-colored animals can also be found (17). Zavot cattle are a valuable genetic resource for Turkey. Thus, the genetic characterization and production traits of this breed should be examined to prevent its extinction. The objective of the present research was to determine the effects of interaction between GH gene polymorphism and meat yield performance in Zavot cattle.

2. Materials and methods

2.1. Animals and DNA samples

This study included a total of 45 male cattle from the Zavot breed. The animals were housed in a feedlot in Kayseri, Turkey, and fed ad libitum with corn silage, hay, and concentrated feed. During the growth performance testing period (30 days from 280 days of age) they received a full concentrate diet ad libitum. All animals were slaughtered at the age of 280 days, after 24 h of fasting. The carcasses were chilled for 24 h at 4 °C. The estimation of slaughter value was based on weight at slaughter, cold carcass weight, and dressing percentage. To determine growth traits, the animals were weighed daily for weight gain. Genomic DNA was extracted using the phenol/chloroform/isoamylalcohol (25:24:1) method from whole blood.

2.2. PCR-RFLP method

Genotyping for GH/*Alu*I polymorphism was performed by PCR-RFLP. The sequences of the forward and reverse primers for the amplification of the GH gene were amplified using the following primers (accession number EF592534.1): forward 5'-GCT-GCT-CCT-GAG-GGC-CCT-TCG-3' and reverse 5'-GCG-GCG-GCA-CTT-CAT-GAC-CCT-3'. PCR for the GH gene was performed in a 25 µL of reaction mixture containing 1.5 mM $MgCl_2$, 200 µM of each dNTP, 200 µM of each primer, 1X PCR buffer, 1 U of Taq polymerase, and 100 ng of genomic DNA template. The reaction mixture was placed in a MyGenie 96 thermal cycler (Bioneer Inc., South Korea). Thermal cycling conditions included an initial denaturation step at 95 °C for 4 min. This was followed by 35 cycles of 40 s each at 94 °C, 60 °C, and 72 °C, and a final extension at 72 °C for 5 min. PCR products were digested for a minimum 3.5 h at 37 °C with 10 U of *Alu*I restriction endonuclease (MBI Fermentase). PCR products and restriction fragments were electrophoresed in 3% agarose gels and stained with ethidium bromide.

2.3. Statistical analysis

Data from the 45 animals were included in the analysis. Statistical analysis was performed by analysis of covariance (ANCOVA) using the GLM procedure. The proposed ANCOVA model is:

$$Y_{ij}=\mu+\alpha_j+\beta(\times_{ij}-\bar{\times})+\varepsilon_{ij}$$

In this model, Y_{ij} is the value of live weight for jth observation in the ith level; μ is the overall (constant) mean value of the live weight; α_j is the effect of ith genotype; β is a combined regression coefficient; $\times_{ij}$ is the covariate (initial weight) value for jth replicate observation from the ith level; $\bar{\times}$ is the overall mean value of the initial weight; and ε_{ij} is random or unexplained error.

ANCOVA is used to increase precision of yield traits in livestock. However, yield traits are applied for factors

that are observational and for measured covariates, and so it may not be biologically meaningful to calculate yield means adjusted to a common value of the covariate. For example, when examining cattle trials, it may not be meaningful to compare the live weight of LL, LV, and VV genotypes adjusted to a common initial weight, because the genotypes have a naturally different mean of initial weight. The fixed effects of genotype and covariates (initial weight) were included in the initial model for all examined traits. Moreover, mean differences of initial weight, carcass weight, and carcass yield among genotype groups were assessed by analysis of variance (ANOVA). All statistical analyses were performed using SPSS 14.01 (SPSS Inc., Chicago, IL, USA).

3. Results

Daily live weight gain in the examined Zavot cattle was observed during fattening. Live weight values at different days of fattening (30 days from 280 days of age) are shown in Figure 1.

The following DNA restriction fragments were expected for GH/*Alu*I polymorphism: 223 bp (not digested) for VV genotype, 171 and 52 bp for LL genotype, and 223, 171, and 52 bp for VL genotype (Figure 2). The smallest fragment (52 bp) of L allele was not observed. However, VV genotype and VL genotype could be detected with a fragment of 171 bp.

The 2 alleles for GH locus were observed in Zavot cattle (L and V), and the L allele (0.77) frequency was found to be higher than the V allele frequency (0.23). The expected frequencies of the 3 genotypes were 26.45 (LL), 2.45 (VV), and 16.1 (LV). The observed number of genotypes 31 (LL), 7 (VV), and 7 (LV) were different to the expected values. Based on the observed vs. expected genotype frequencies, the entire pool was not in Hardy–Weinberg genetic equilibrium ($P < 0.001$). The GH-L allele was predominantly found in Zavot cattle (Table 1).

Initial weight of different GH genotypes is shown in Table 2. Initial weight was higher in Zavot cattle with LL genotype compared to those with LV and VV genotypes, but the effect of genotypes on initial weight was found to be nonsignificant ($P > 0.05$).

In this study, hypothesis H_0 suggested that the effect of genotype on live weight is not significant. H_0 hypothesis was accepted in all live weight ($P > 0.05$). In ANCOVA, the initial weight was taken as a covariate that was closely related to live weight. Using the 30–150 day live weight, the covariates were found to be significant enough to predict the dependent variable. However, the 180–280 day live weight was not significant. Accordingly, changes in initial weight of 1 unit increased the live weight by 1.07, 0.97, 0.78, 0.70, and 0.63 units on days 30, 60, 90, 120, and 150, respectively. The adjusted means for all analyzed traits are given in Table 3. After adjusting the effect of the covariate, the genotype effect was not found to be significant for all live weight ($P > 0.05$).

The results of carcass traits are given in Table 4. The differences between the means of genotype groups were not found to be significant for carcass weight and carcass yield ($P > 0.05$).

4. Discussion

Beef cattle are slaughtered between 6 and 12 months of age. Several features, such as carcass yield, meat quality, and meat composition, can be only detected after slaughtering. Therefore, marker-assisted selection has become

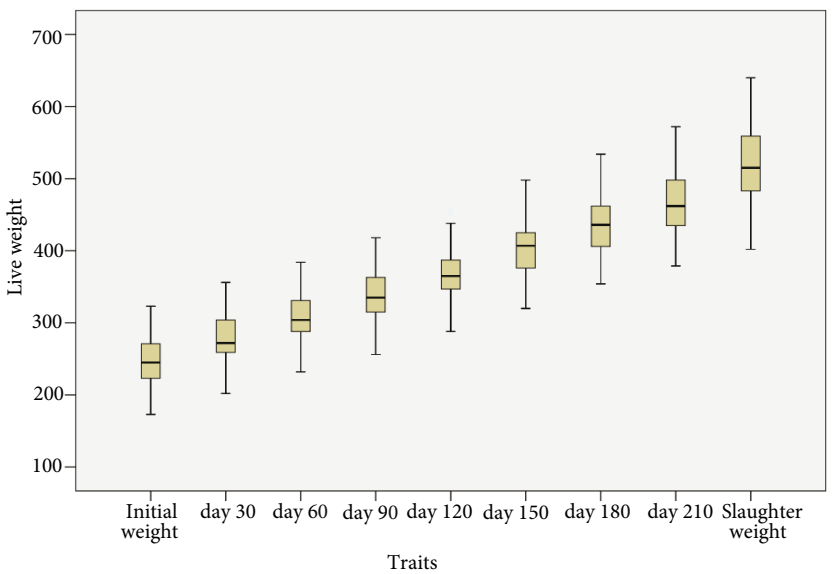

Figure 1. Live weight of 45 male Zavot cattle on different days of fattening.

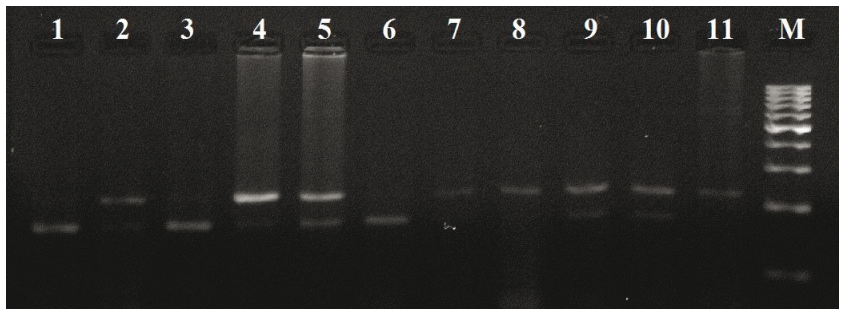

Figure 2. GH genotyping after digestion with *Alu*I restriction enzyme. M DNA marker (100 bp); lanes 1, 3, and 6: genotypes LL; lanes 7, 8, and 11: genotypes VV; lanes 2, 4, 5, 9, and 10: genotype VL.

Table 1. Frequency of alleles and genotypes of GH/*Alu*I polymorphism.

	GH-*Alu*I polymorphism					
Frequency	Genotype			Allele frequency		Statistical significance (Chi-squared HWE)
	LL	VV	LV	L allele	V allele	
Observed	31	7	7	0.77	0.23	$X^2 = 14.38$
Expected	26.45	2.45	16.1			$P < 0.001$ (df = 1)

HWE: Hardy–Weinberg Equilibrium; df: degree of freedom.

Table 2. Means, standard errors of means (SEM), and statistical significance for initial weight.

Traits	Genotype	N	Mean ± SEM	Statistical significance (ANOVA)
Initial weight (kg)	LL	31	248.03 ± 5.87	F: 0.42; P > 0.05
	VV	7	234.86 ± 17.59	
	LV	7	241.57 ± 14.69	

increasingly important in livestock in recent years. Genetic markers and their polymorphisms have attracted much attention as a tool for genetically improving farm animals (13). Several genes, such as encoding for GH, GH receptor, insulin-like growth factor-I, and prolactin hormone, are thought of as candidate markers to determine quantitative traits in farm animals. Researchers suggest that these genes could assist progress in the genetic selection of livestock (6). However, studies on genetic markers have shown contradictory results. Consequently, more research should be conducted to determine the relationship between genetic markers and yield traits in livestock. In addition, these studies should be repeated with different livestock breeds of the same species. A limited number of studies have examined the effect of these genes on growth performance, weight gain, and carcass yield traits in cattle (6).

Growth hormone acts directly by binding its receptors to bone, muscle, and fat tissue cells, and induces cell proliferation in these tissues. In addition, GH increases muscle protein intake and affects mammary growth in mammalians. Moreover, it plays an indirect role in cell growth (5). Therefore, GH polymorphism has been considered by researchers to affect weight gain, carcass weight, birth weight, and carcass quality such as marbling (12,18). Due to the crucial role of GH in lactation and animal growth, the GH gene is thought to be a candidate marker for performance traits in livestock animals such as cattle.

The GH/*Alu*I polymorphism consists of a cytosine–guanine replacement at codon 127 in this gene. Therefore, the substitution of leucine (L allele) by valine (V allele) leads to the appearance of 3 genotypes of the GH gene and produces different physiological processes (19).

Table 3. Means (no covariate used)/adjusted means ($\bar{y}_{i(ad)} = \bar{y}_{ij} - b(\bar{x}_i - \bar{x})$), their standard errors (SEM), and statistical significance for live weight.

Live weight	Genotype	N	Unadjusted mean ± SEM	Adjusted mean ± SEM*	Statistical significance (ANCOVA)
Day 30	LL	31	280.06 ± 5.81	277.17 ± 1.54	Initial weight effect: F: 600.7; P < 0.001 Genotype effect: F: 1.21; P > 0.05 Interaction: F: 1.42; P > 0.05 R^2: 0.95
	VV	7	270.29 ± 18.90	281.16 ± 3.31	
	LV	7	273.71 ± 15.92	277.37 ± 3.24	
Day 60	LL	31	310.10 ± 6.14	307.15 ± 2.19	Initial weight effect: F: 282.1; P < 0.001 Genotype effect: F: 0.26; P > 0.05 Interaction: F: 0.44; P > 0.05 R^2: 0.90
	VV	7	303.14 ± 19.14	314.09 ± 4.72	
	LV	7	304.57 ± 14.68	307.88 ± 4.62	
Day 90	LL	31	339.10 ± 6.59	336.07 ± 3.03	Initial weight effect: F: 138.3; P < 0.001 Genotype effect: F: 1.07; P > 0.05 Interaction: F: 1.31; P > 0.05 R^2: 0.83
	VV	7	335.43 ± 20.63	347.08 ± 6.52	
	LV	7	334.57 ± 12.75	337.23 ± 6.38	
Day 120	LL	31	368.29 ± 7.05	365.22 ± 3.82	Initial weight effect: F: 80.9; P < 0.001 Genotype effect: F: 0.96; P > 0.05 Interaction: F: 1.06; P > 0.05 R^2: 0.75
	VV	7	367.29 ± 20.30	378.49 ± 8.21	
	LV	7	365.57 ± 12.67	367.95 ± 8.03	
Day 150	LL	31	402.74 ± 7.60	399.72 ± 4.96	Initial weight effect: F: 44.2; P < 0.001 Genotype effect: F: 0.70; P > 0.05 Interaction: F: 0.75; P > 0.05 R^2: 0.63
	VV	7	404.86 ± 20.02	415.76 ± 10.68	
	LV	7	401.43 ± 14.68	403.60 ± 10.45	
Day 180	LL	31	433.23 ± 8.03	430.24 ± 5.70	Initial weight effect: F: 32.3; P < 0.001 Genotype effect: F: 0.46; P > 0.05 Interaction: F: 0.50; P > 0.05 R^2: 0.56
	VV	7	441.29 ± 19.54	451.62 ± 12.16	
	LV	7	430.86 ± 15.51	433.01 ± 11.89	
Day 210	LL	31	462.29 ± 8.46	459.39 ± 6.45	Initial weight effect: F: 22.5; P < 0.001 Genotype effect: F: 0.55; P > 0.05 Interaction: F: 0.59; P > 0.05 R^2: 0.48
	VV	7	473.29 ± 19.88	483.69 ± 13.88	
	LV	7	462.43 ± 16.64	464.23 ± 13.57	
Day 280 (Slaughter weight)	LL	31	518.10 ± 9.80	515.62 ± 8.54	Initial weight effect: F: 8.29; P < 0.01 Genotype effect: F: 0.98; P > 0.05 Interaction: F: 1.07; P > 0.05 R^2: 0.31
	VV	7	540.29 ± 21.08	551.09 ± 18.38	
	LV	7	527.00 ± 19.21	527.47 ± 17.98	

*Covariates appearing in the model are evaluated at the following values: Initial weight = 244.98 kg
Interaction: interaction between initial weight and genotype effect
F: F statistic for the degree main effect
P: P value (the observed significance level)
R^2: coefficient of determination.

Consequently, it has been reported that the GH gene may be an indicator for meat production traits, denoting that it could be used for the genetic improvement of beef cattle (12). Nevertheless, it has also been reported that the polymorphisms at GH are not a sufficient tool for the selection of beef cattle. Furthermore, it has been stated that additional research on the relationship between GH gene polymorphism and yield traits is necessary (5), because the results of studies on the relationship between GH gene polymorphism and yield traits were conflicting. Some investigators reported a relationship between this gene and meat yield traits in different cattle breeds. For example, a significant association was found between GH locus, weight gain, and body weight at slaughter in Zebu and Zebu crossbreeds, such as Canchim (19). Similarly, this association has been reported to be different in *Bos taurus* cattle breeds such as Simmental, Japan Black cattle, and Slovakian Pied cattle (11,20–23). In contrast, Di Stasio et al.

Table 4. Means, standard errors of means (SEM), and statistical significance for carcass weight and yield.

Carcass traits	Genotype	N	Mean ± SEM	Statistical significance (ANOVA)
Carcass weight (kg)	LL	31	301.12 ± 5.96	F: 0.280; P > 0.05
	VV	7	293.63 ± 10.61	
	LV	7	307.43 ± 10.50	
Carcass yield (%)	LL	31	0.58 ± 0.001	F: 0.109; P > 0.05
	VV	7	0.57 ± 0.01	
	LV	7	0.58 ± 0.01	

(24) reported no association between GH polymorphism, growth, and carcass traits. Nevertheless, some investigators have reported that the LL genotype is superior to other genotypes (15,19,20). Conversely, it has been reported that heterozygous genotype animals are significantly superior to LL and VV genotypes in terms of carcass gain and meat value (22). Our results also showed no significant effect of GH/*Alu*I polymorphism on live weight gain and meat production in Zavot cattle.

On the other hand, studies exist on the effects of GH on different traits, except for meat production. For example, a significant association was found between calf birth weight and GH genotypes in the Holstein breed (18). However, no significant association was found between GH genotypes and birth weight in Jersey and crossbred cattle (18). Another study found a relationship between GH genotypes and GH secretion (21). These conflicting results can be explained by differences in the linkage disequilibrium between markers and quantitative trait loci in the various cattle breeds, by different epistatic interactions between the genetic bases of the examined breeds and QTL, and by the different design of the experiments and statistical methods used (19).

In conclusion, the GH/*Alu*I polymorphism is not yet sufficient for use in beef cattle selection programs. Further research should be conducted on the relationship between GH gene polymorphism and yield traits in different cattle breeds. The mutual effects of GH and other markers on meat yields should also be investigated.

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