The effect of environmental factors and Growth Hormone Receptor gene polymorphism on growth curve and live weight parameters in buffalo calves

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Abstract: This study was conducted to determine the effects of some environmental factors and growth hormone receptor gene polymorphisms (GHR) on Brody growth curve coefficients and different body weights parameters in buffalo calves. The records and blood samples of 27 buffalo calves, born in 2009, bred at Afyon Kocatepe University Animal Research Center were analyzed. A, B and k parameters for the Brody model were estimated as 366.589±31.698, 0.932±0.011 and 0.002±0.000 respectively. In addition, live weights at birth, 180 and 360 days were 30.696±1.043 kgs, 121.701±5.071 kgs and 188.834±8.442 kgs respectively. Three polymorphic regions were detected at G3676A, G3679A and G3680del bases in exon 10 of the GHR gene. Effects of sex on parameter A and birth weight on weight at 180 days were statistically significant (P<0.05). Findings indicated that G3676A and G3679A mutations and haplotypes consisting of mentioned SNPs may result in significant differences in growth traits, but the available data were not enough to reveal this fact.

Key words: Water buffalo, growth curve, growth hormone receptor, SNP

Çevresel faktörler ve Büyüme Hormon Reseptörü genindeki polimorfizmin malaklarda büyüme eğrisi ve canlı ağırlık parametrelerine etkisi

Özet: Bu araştırma, malaklarda Brody büyüme eğrisi katsayılarına ve farklı canlı ağırlık parametrelerine çeşitli çevre faktörleri ve büyüme hormon reseptör (GHR) genindeki polimorfizmin etkilerini belirlemek amacıyla yapılmıştır. Araştırmanın materyalini Afyon Kocatepe Üniversitesi Hayvancılık Araştırma ve Uygulama Merkezinde yetiştirilmiş 2009 doğumlu 27 baş malağa ait kayıtlar ve kan örnekleri oluşturmuştur. Malaklarda eğri katsayıları A, B ve k ile doğum, 180. gün ve 360. gün canlı ağırlıkları sırasıyla 366.589±31.698, 0.932±0.011, 0.002±0.000, 30.696±1.043 kg, 121.701±5.071 kg ve 188.834±8.442 kg olmuştur. GHR geninin 10. ekzonunda G3676A, G3679A ve G3680del olmak üzere üç polimorfik bölge tespit edilmiştir. A katsayısı üzerine cinsiyetin ve 180. gün ağırlığı üzerine doğum ağırlığının etkisi önemli (P<0,05) bulunmuştur. Bulgular, G3676A ve G3679A pozisyonlardaki polimorfizm ile haplotiplerin önemli farklara yol açma eğiliminde olduğunu fakat eldeki verilerin bunu ortaya koymaya yetmediğini göstermiştir.

Anahtar kelimeler: Manda, büyüme eğrisi, büyüme hormon reseptörü, SNP

Introduction

Growth is an important factor as associated with yield and growth characteristics of farm animals including buffaloes. Because of the trait is affected by various environmental and genetic factors to reveal in the progress of growth is difficult [6]. Nonlinear functions are used to describe the growth by tangible and interpretable curve parameters [2]. The use of growth curves in animal husbandry has been accelerated by Brody (1945)'s researches [1]. Giving desired shape to growth curves is aimed in some researches of genetic improvement [28]. The effect of growth hormone depends on the interaction with related receptors (GHR) on the surfaces of target cells [10]. Some changes in the functional regions of the growth hormone receptors have potential to affect the signal pathway and binding capacity. The activity of growth hormone in target tissues can be altered by this way [15]. Binding between growth hormone and GHR leads to dimerization, activation of the tyrosine kinase associated with GHR, and tyrosine phosphorylation of both Janus kinase 2 (JAK2) and GHR [29]. Activations of the growth determining signal molecules such as mitogen-activated protein kinase (MAPK), signal transducer and activator of transcription (STAT) and protein kinase C (PKC) transcription factors are initialized by these

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reactions [14]. All the processes are prone to be effected by genetic polymorphism. Selection through the genetic markers can be used to accelerate the improvement in traits of economic importance for animal production [16]. Studies were generally focused on the identification of single nucleotide polymorphism (SNP). Declination of SNPs causing differences in economic traits can be expected due to intensive selection. However, the number of SNPs affecting economic traits are still exceeding other types of genetic markers [11]. Excessive number of SNPs in the genome makes them a powerful tool for genetic studies. More accurate estimates of breeding values might be possible by identification of loci affecting production traits and use of molecular markers. Relationships between SNPs and economic traits importance have been reported in cattle research [6,13,19].

In this study, it was aimed to determine the effects of various environmental factors and the polymorphisms in growth hormone receptor (GHR) gene on Brody growth curve coefficients and different live weight parameters in buffalo calves.

Materials and Methods

The material of this research consisted from records and blood samples of 27 buffalo calves born in 2009 within the scope of a Buffalo Project [23] conducted at Afyon Kocatepe University Animal Research Center. Buffalo calves were weighted with a 500 grams sensitivity scale for every month through the year. Live weight and age were taken as dependent and independent variables and Brody model ($Y_t = A (1-B.exp^{-k.t})$) were analyzed using the computer program NLREG [22]. In the model, Y_t is observed live weight in t^{th} age (days), A is adult live weight, B is integral constant and k is a function of the ratio of maximum growth rate to mature weight or maturing rate [4,7,20]

In this study, blood samples of 27 buffalo calves were collected aseptically by venous puncture from Vena jugularis to anticoagulant (EDTA) vacutainers. DNAs from blood samples were isolated according to standard phenol–chloroform methods [18]. DNA concentrations were determined using a Multiskan GO µdrop spectrophotometer (Thermo Scientific) and adjusted to 25 ng/µl. DNA products were either amplified immediately or stored at -35°C. On the basis of available GHR gene exon 10 sequence (Genbank: EF207441.2), buffalo gene-specific primers were designed using FastPCR software [12]. The primers were forward 5'-CTACAATGATGACTCTTGGGTTGAA-3' and revers 5'-TGCCACTAAACAGTCTTTGAGAC-3'. The initial PCR mixture consisted of 2 µl DNA, 10 mM of each primer, 2,5 µl of 10xPCR buffer with 2mM $MgCl_2$ (Thermo), 200mM of dNTPs, 1 unit of Phusion Taq DNA Polymerase (Thermo) and 5.0µl Q-Solution (Qiagen) in a total volume of 25 µl. Thermal cycling was performed using Veriti thermal cycler (Applied Biosystems) starting with 10 min at 95°C, followed by 35 cycles of 30 sec at 95°C, 30 sec at 64°C, and 30 sec at 72°C, and a final extension for 5 min at 72°C. Amplicon yields were evaluated by running 3 µl of PCR product on a 2% agarose gel and visualized with GelRed nucleic acid gel stain (Biotium) and a VisionCapt (Bio-Vision, Vilber Louramat) imaging system. PCR products were cleaned with ExoSAP (Thermo). The cycle sequencing reaction consisted of 1µl 5xCycle sequencing buffer, 1µl ABI PRISM Big Dye Terminator Cycle Sequencing Ready Reaction Kit v3.1, 1 µl sequencing primer (10 pmol), and 1 µl amplification product for a total volume of 7 µl. The cycle sequencing program consisted of 35 cycles of denaturation at 96° C for 10 sec, primer annealing at 57°C for 15 sec, and extension at 60°C for 4 min, and the reaction was run in a Veriti thermal cycler (Applied Biosystems). The cycle sequencing products were ethanol precipitated and analyzed on an ABI 3500 DNA sequencer (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. DNA sequences were edited in Sequencher 4.1 (Gene Codes Corporation, Ann Arbor, MI, USA). The consensus sequences were further aligned in BioEdit 7.2.0 [9]. The polymorphisms were analyzed as numbers of alleles and observed (H_o) heterozygosity. These values were obtained using the GENETIX 4.03 program [5].

Effects of birth month, age of dam, origin, gender and genotype on coefficients of Brody function and birth weight were analyzed by using individual records and genetic data.

The following model was used:

Yijklmn= µ++DAi+AYj+Ok+Cl+GENm+eijklmn

where Yijklmn is the observed trait of interest; µ is the overall mean; DAi is the effect of the birth

month (group 1 for 4-8th months, group 2 for 9-12th months); AYj is the effect of the age of dam (group 1 for 6-12 ages, group 2 for 13-22 ages); Ok is the effect of the origin (Afyonkarahisar province, Çorum province, Diyarbakır province and Bandırma Sheep Research Station); Cl is the effect of gender (male, female); GENm is the effect of polymorphism in GHR gene (G3676A, G3679A, G3680del for SNPs; α, β, γ, δ for haplotypes) and eijklmn is the random error N (0,σ2).

Weights at six and twelve months old calves were estimated by interpolation [8] and the birth weight was added to model as a discrete factor for them. Birth weights were grouped up to 31 kgs (group 1) and greater than and equal to 31 kgs (group 2). PASW Statistics 18 computer program [3] was used for analysis of variance.

Results

Three SNPs were identified at 3676, 3679 and 3680 bases of exon 10 of the GHR gene. First two of them were not reported in literature by now. The allele frequencies and heterozygosity values of the SNPs and haplotypes are shown in Table 1.

Table 1. The allele frequency and heterozygosities in all SNPs and haplotypes.

*: SNPs are numbered according to the nucleotide position on sequence obtained from GeneBank (accession EF207441.2)

SNP*	Allele Frequencies (%)		H_o	Haplotype			
				α (alfa)	β (beta)	γ (gama)	δ (delta)
G3676A	A (0.20)	G (0.80)	0.32	GG	GG	GG	AA
G3679A	A (0.11)	G (0.89)	0.20	GG	GG	GA	GG
G3680del	G (0.28)	Del (0.72)	0.40	GG	Del	Del	Del

Table 2. ANOVA results and least square means for the effects of different factors and polymorphism at base 3676.

	n	A	B	k	Birth Weight	180 day Weight	360 day Weight
μ	27	366.589±31.698	0.932±0.011	0.002±0.000	30.696±1.043	121.701±5.071	188.834±8.442
Environmental Effect							
Birth Month							
April-August	10	399.336±44.155	0.947±0.015	0.002±0.000	30.410±1.453	125.649±7.120	197.802±11.851
September-December	17	333.842±36.008	0.916±0.012	0.002±0.000	30.981±1.185	117.754±5.595	179.866±9.313
Age of Dam							
6-12	12	409.015±36.383	0.935±0.012	0.002±0.000	29.559±1.198	122.159±5.919	191.027±9.853
13-22	15	324.163±47.297	0.929±0.016	0.002±0.000	31.833±1.557	121.244±7.348	186.641±12.232
Origin							
Afyon	6	399.041±67.390	0.938±0.023	0.002±0.001	31.138±2.218	132.469±10.921	206.921±18.179
Bandırma KAİ	3	244.836±85.852	0.897±0.029	0.002±0.001	30.702±2.826	96.346±13.514	144.439±22.496
Corum	6	404.476±61.263	0.953±0.021	0.003±0.001	29.275±2.017	139.001±10.586	215.869±17.621
Diyarbakır	12	418.003±39.475	0.939±0.013	0.002±0.000	31.667±1.299	118.990±6.100	188.106±10.154
Sex		*				*	
Male	18	426.326±33.920	0.943±0.012	0.002±0.000	31.256±1.117	130.462±7.350	200.275±8.753
Female	9	306.852±46.540	0.921±0.016	0.003±0.000	30.135±1.532	112.941±5.661	177 .392±12.516
Birth Weight							
<31 kg	14	-	-	-	-	126.101±8.601	176.792±9.423
≥31 kg	13	-	-	-	-	116.873±9.591	200.876±12.236
Individual SNP Effect							
G3676A							
G	21	316.111±39.364	0.921±0.013	0.002±0.000	30.559±1.296	118.170±6.081	179.402±10.122
A	6	417.067±62.015	0.942±0.021	0.002±0.001	30.832±2.041	125.232±9.936	198.266±16.540

*: P<0.05

Table 3. ANOVA results and least square means for bases 3679 and 3680 and different haplotypes.

	n	A	B	k	Birth weight	180 day weight	360 day weight
Individual SNP effect							
G3679A							
G	24	358.039±30.935	0.928±0.010	0.002±0.000	30.214±0.953	120.753±4.925	186.805±8.266
A	3	291.356±77.430	0.929±0.026	0.003±0.001	33.604±2.387	118.445±12.088	177.267±20.289
G3680del							
Del	21	349.432±30.409	0.929±0.010	0.002±0.000	30.711±0.962	120.356±4.601	185.405±7.757
G	6	349.834±70.513	0.919±0.023	0.002±0.001	30.097±2.230	120.999±11.052	185.068±18.631
Haplotype effect							
Haplotype							
α (alfa)	6	323.290±73.907	0.923±0.024	0.002±0.001	30.894±2.265	120.823±11.727	183.801±19.565
β (beta)	13	320.680±45.542	0.916±0.015	0.002±0.000	29.924±1.396	116.846±7.102	177.524±11.848
γ (gama)	2	254.802±105.474	0.968±0.034	0.003±0.001	35.814±3.232	124.330±17.620	186.583±29.397
δ (delta)	6	419.872±65.034	0.941±0.021	0.002±0.001	30.677±1.993	124.989±10.735	198.192±17.910

The coefficient of determination (R2) for Brody model was 0.98. ANOVA results for the effects of different environmental factors and polymorphism at base 3676 are presented in Table 2. Same procedures were conducted for SNPs at positions 3679 and 3680, and different haplotypes and only polymorphic effects are given in Table 3.

Discussion

In the study, parameter A was calculated as 366.589 kgs. This was lower than values reported by Salem et al. [17] The effect of gender on A was significant ($P < 0.05$) and males and females were 426.326 and 306.852 kgs. Gomez et al. [7], Aroujo et al. [4], Torres et al. [25] and Şahin et al. [20] announced higher values than these findings. Differences can be caused by some factors such as breed, measurement interval and age at last measurement. Least square means of weight at 180 days age for male and females were 130.462 and 112.941 kgs. This trait was also significantly ($P < 0.05$) effected by gender. These findings were higher than the literatures [21, 24, 26]. This variation may be due to differences in breed and feeding and management conditions.

Amplified sequencing analysis was performed for the exon 10 of growth hormone receptor gene by PCR. Vijh et al. [27] (Genbank: EF207441.2) have found A2767G, A2770G, A2838G, A3079G, C3630T and G3679del at exon 10 of GHR gene in water buffaloes. Three polymorphic regions (G3676A, G3679A and G3680del) were determined in this research. First two of them were new in water buffaloes. Vijh et al. [27] have also identified polymorphic mutations at SNPs A3149G, T3239C and C3353T. Unlike these SNPs were determined monomorphic in this research.

In this study, significant differences could not be found for polymorphisms at nucleotide positions 3676, 3679 and 3680 and haplotypes. However, the perusal of the table-3 showed that some polymorphisms have a tendency to lead to some significant differences for parameter A and live weight at 180 and 360 days. But the available data were not enough to detect the differences.

As a result, it was concluded that there is a need for further studies to be carried out and whether polymorphisms at bases 3676 and 3679 and haplotype δ may be used in a selection program.

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