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Marker-assisted introgression of myostatin from Texel to Ramlıç sheep: Growth and real-time ultrasound carcass traits in F_1 and BC_1 lambs

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Abstract: The aim of the study was to evaluate live weights, body measurements, and real-time ultrasound carcass traits of F_1 and BC_1 genotypes in a marker assisted introgression (MAI) process. Effects of some factors on live weight and body measurements including ultrasonographic assessments in *Musculus longissimus dorsi* (MLD) were investigated at the F_1 (n=51) and BC_1 (n=99) cross lambs born in 2015 – 2017 period. Variance analysis showed that genotype had significant ($P < 0.001$) effect on pre- and post-weaning growth characteristics. The birth type had significant ($P < 0.05$) effect on pre- and post-weaning growth traits. Live weights at twelve months of age were higher in heavier born lambs. The growth performance of F_1 lambs was between Texel and Ramlıç. Myostatin heterozygous BC_1 lambs had been potentially heavier than the non-carriers and pure Texel. MLD depth and area were also statistically higher ($P < 0.05$) in BC_1 lambs carrying myostatin than non - carriers. As a result, the beginning phase of introgression processes implemented was found to be successful.
Keywords: Crossing, introgression, myostatin, Ramlıç, Texel.

Miyostatin mutasyonunun Teksellerden Ramlıçlara belirteç yardımlı aktarımı: F_1 ve G_1 kuzularda büyüme ve gerçek zamanlı ultrasonografik karkas özellikleri

Özet: Bu çalışma, miyostatin mutasyonunun belirteç yardımlı aktarım yöntemi ile Teksellerden Ramlıç koyunlarına geçirilmesi sırasında F_1 ve G_1 kuzularda büyüme ve ultrasonografik karkas özelliklerindeki değişimi belirlemek amacıyla yapılmıştır. 2015 - 2017 sezonlarında doğan F_1 (n = 51) ve G_1 (n = 99) kuzularda canlı ağırlık, vücut ölçümleri ve belgözü kasının (*Musculus longissimus dorsi*) ultrasonografik değerlendirmeleri üzerine kimi faktörlerin etkileri araştırılmıştır. Varyans analizi sonuçları, genotipin sütten kesim öncesi ve sonrası büyüme özellikleri üzerinde önemli (P<0,001) bir etkiye sahip olduğunu göstermiştir. Sütten kesim öncesi ve sonrası büyüme özellikleri üzerine doğum tipinin istatistiki olarak önemli (P<0,05) olduğu saptanmıştır. Doğum ağırlığı yüksek olan kuzuların 12. ay canlı ağırlığı da yüksek olmuştur. F_1 kuzularda büyüme performansı bulguları Teksel ve Ramlıç kuzular için belirlenen değerler arasında bulunmuştur. Tek kopya miyostatin mutasyonu taşıyan G_1 kuzuların taşıyıcı olmayan G_1 ve saf Teksel kuzulardan ağır olma kapasitesi bulunduğu görülmüştür. MLD derinliği ve alanı bakımından mutasyonu taşıyan G_1 kuzular, taşımayanlara göre önemli (P<0,05) düzeyde üstün bulunmuştur. Sonuç olarak uygulanan introgresyon programının başlangıç aşamasının başarılı olduğu görülmüştür.
Anahtar sözcükler: İntrogresyon, melezleme, miyostatin, Ramlıç, Teksel.

Introduction

The Ramlıç sheep carrying 65% American Rambouillet and 35% indigenous Dağlıç genotypes has become a native breed in the ancient Phrygian region of Anatolia for the last decades. Birth weight, daily live weight gain, weights at weaning and six months of age for

Ramlıç lambs were reported in the ranges of 4.04 - 4.63, 0.270 - 0.300, 22.44 - 30.92 and 29.04 - 37.40 kg, respectively (1, 7, 11, 12, 15, 21, 34). Same traits except for weight at six-months of age for Texel and its crosses in different conditions were found between 3.04 - 5.03, 0.17 - 0.32 and 14.86 - 34.42 kg, respectively (2, 9, 10, 13, 22, 23, 26 - 28, 33). Meanwhile, the live weight in this breed has reached to 45.2 - 46.7 kg at the age of 12 months according to McMillan et al. (28). The depth of Musculus longissimus dorsi in British Texel was reported to be 27.5 mm at 140 days of age according to ultrasonographic measurements by Wolf and Jones (32). Additionally, same researchers observed that the body length, wither height, chest circumference and rump width measurements as 58.0, 55.8, 80.9 and 24.6 cm respectively. The mean live weight obtained in the study was expressed as 43.6 kg. The chest circumference in weaning lambs carrying 50% Texel genotype was also 61.56 cm according to Koritiaki et al. (23). Above mentioned growth characteristics were reported to be affected by different environmental factors such as sex, birth type, month of birth, year, and weaning age (15).

Texels are known as homozygous carriers of myostatin (Mstn +/+) mutation leading to the double muscling phenotype in sheep market throughout the World, while Ramlıç is one of the meat - wool sheep breeds in Turkey. Breeding of leaner and fast-growing lambs and the production of high - quality carcasses are key components to compensate for the meat production deficiency in Turkey. Genetic progress can be accelerated by identifying and using the Quantitative Trait Loci (QTL) or Nucleotides (QTN) in marker - assisted selection (19). Myostatin mutation (g+6723G>A) reported in Texel sheep is located in 3'untranslated regions of Growth differentiation factor 8 gene (*GDF8* or *Myostatin*) (8, 24). Valuable alleles such as myostatin are transferred from foreign breeds to natives by introgression which is the procedure whereby a marker gene is swapped. Thus, a new allele will have been added to the gene pool of indigenous breed (25, 31). This method has been used to pass the Myostatin allele to Lacaune breed successfully Grasset et al. (16). This mutation can be used to increase muscle development and growth of lambs in the selection programs of developing countries.

The aim of the study was to evaluate the growth characteristics of F_1 and BC_1 genotypes during the transfer of myostatin mutation (g+6723G>A) from Texel into the Ramlıç by introgression.

Materials and Methods

In this study, animals were treated following the guidelines of the experimental animal ethics committee of the Afyon Kocatepe University (Decision no.49533702-26). This research was carried out in Afyonkarahisar province Sheep and Goat Breeders Association's Stud Animals Breeding and Test Station situated at 39°02'59.3"N 31°20'23.9"E.

Eight myostatin homozygous pure Texel rams were mated with pure Ramlıç ewes to produce F_1 (Mstn +/-) (Texel X Ramlıç) lambs (n = 51) in the summer of 2015. Then, a total of 100 pure Ramlıç ewes were bred with F_1 ram lambs (n = 20) carrying single - copy myostatin. BC_1 (F_1 X Ramlıç) lambs (n = 99) were born in 2016 - 2017 lambing season.

Eighty-eight BC_1 lambs were genotyped to detect carriers or non - carriers for the aspect of myostatin mutation. The weaning was realized after the overwhelming majority of lambs arriving the age of 120 days. The non - carrier BC_1 lambs were culled after weaning according to the selection program conducted on the farm. Daily live weight gain was estimated using the birth and weaning weights. Also, the weights at six and 12 months of age were calculated by interpolation from the weights recorded periodically.

Records at the time of weaning for some body measurements (wither height, body length, rump width and heart girth) and ultrasonographic values (area and depth) of *Musculus longissimus dorsi* (MLD) and backfat thickness (BF) in F_1 and BC_1 lambs were also used to evaluate the progress in the project.

The ultrasonographic inspections (Mindray DP10, China) were carried out from the midst of 12 and 13th ribs with a 5 MHz convex probe (23). The images were recorded with the MP4 player (Orite® PMP500, Australia) connected to the USG via cable and transferred to a computer. These images were monitored with GOM Player (Gretech Corporation, South Korea) and frozen at the most appropriate time and measured using ImageJ software (National Institute of Health, Bethesda, USA). ImageJ values were calibrated before measurements as described by Bracken et al. (5).

Blood samples for genetic analyses were collected from the *Vena jugularis* of the animals to vacuum tubes with EDTA and were brought to the laboratory in the cold chain and kept at + 4ºC until the DNA isolation stage. DNA extraction was made with a modified method described by Boom et al. (4). DNA samples were checked for their integrity on the agarose gel and their amounts were measured using a spectrophotometer. DNA quantity and quality were controlled and stored at –20 ° C.

g+6723_F_5'-GGT TCG TGA TGG CTG TAT AAT GTG A-3' and g+6723_R_5'-GAT TTC AGA TAA TAG AGT TAA ATC ATT TTG GTT TGC TT-3' primers designed with FastPCR 6.1.2 program (20) were used to determine the SNP (g+6723G>A) in the 3'UTR region of GDF8 gene according to NC_019459.2 reference sequence of NCBI (National Center for Biotechnology Information). A region of 136 bp is amplified with PCR.

Then, a band of 136 bp for AA genotype, 136 and 68 bp bands for AG genotype and 68 bp band for GG genotype were obtained by cutting the PCR product with Tail Restriction Enzyme. For this purpose, a total of 10 µl PCR mixture including 1 µl genomic DNA, 0.8 µl $MgCl_2$ (50 mM), 0.25 µl dNTP, 0.25 µl for each primer (10 pmol each), and 0.0625 µl Platinium Taq Polymerase (Thermo) were prepared. Reactions were performed on the ABI Veriti PCR instrument. Amplification was carried out by using denaturation for 120 seconds at 95 °C and 40 cycles of 95 °C for 30 seconds, 60 °C for 30 seconds and 72 °C for 60 seconds. PCR samples were confirmed with 2% agarose gel electrophoresis. For RFLP, a mix consisted of 5 µl PCR product, 1 µl 10x FastDigest Green buffer, 0.5 µl FastDigest *Tai*I restriction enzyme (Thermo, FD1144) and 3.5 µl ddH_2O incubated for 15 minutes at 65 °C and monitored on 2% agarose gel electrophoresis for genotyping.

Data were analyzed by GLM procedure of MINITAB 18 (29) with the following statistical models: $Y_{ijklmn} = \mu + G_i + S_j + BT_k + BM_l + DA_m + e_{ijklmn}$ for birth weight and daily live weight gain; $Y_{ijklmnop} = \mu + G_i + S_j + BT_k + BM_l + DA_m + WA_n + BW_o + e_{ijklmnop}$ for weaning weight, weight at six months of age, weight at 12 months of age, morphometric measurements and ultrasonographic MLD characteristics at weaning.

Where Y = observation value (morphometric and ultrasonographic measurements), µ = overall mean, G = effect of genotype (Ramlıç, F_1, BC_1 (+/-), BC_1 (-/-) and Texel),

S = effect of sex (male and female), BT = effect of birth type (multiple or single), BM = effect of birth month (December, January and February), DA = effect of dam age (≤730 d, >730 and ≤1095 d and >1095 d), WA = effect of weaning age (≤130 d, >130 and ≤145 d and >145 and ≤180 d), BW = effect of birth weight (≤4.5 kg and >4.5 kg) and e = random error N (0, σ^2) related with each observation. The Tukey procedure provided by in Minitab 18 (29) was used for multiple comparisons.

Results

The least squares means for morphometric and ultrasonographic measurements of Ramlıç, F_1, BC_1 and Texel lambs are presented in the Tables 1, 2, 3 and 4.

Table 1. Least squares means for growth traits of Ramlıç, F_1 and Texel lambs born in 2015 - 2016.

Factor		n	Birth weight (kg)	Average daily weight gain (kg)	Weaning weight (kg)	n	Weight at six mo. of age (kg)	n	Weight at twelve mo. of age (kg)
	µ	143	3.870±0.153	0.17460±0.00577	30.488±0.930	126	34.382±0.944	106	49.03±1.47
Genotype			***	***	***		***		***
	Ramlıç	21	4.100±0.252[a]	0.18760±0.00950[a]	33.89±1.550[a]	19	39.360±1.560[a]	19	59.07±2.39[a]
	F_1 (+/-)	51	4.147±0.159[a]	0.18868±0.00600[a]	30.013±0.869[b]	47	34.403±0.862[b]	42	50.41±1.34[b]
	Texel (+/+)	71	3.364±0.189[b]	0.14751±0.00712[b]	27.56±1.190[b]	60	29.380±1.240[c]	45	37.61±2.04[c]
Sex			**	‡	‡		**		**
	Female	83	3.667±0.171[b]	0.16970±0.00645	29.74±1.03	75	33.12±1.04[b]	66	44.86±1.63[b]
	Male	60	4.073±0.169[a]	0.17950±0.00635	31.24±0.98	51	35.64±1.00[a]	40	53.20±1.59[a]
Birth type			***	***	**				
	Multiple	44	3.569±0.201[b]	0.16177±0.00756[b]	28.93±1.18	40	32.860±1.180	33	47.83±1.85
	Single	99	4.172±0.144[a]	0.18742±0.00544[a]	32.048±0.86	86	35.899±0.887	73	50.23±1.40
Birth month			*	*					
	December	7	3.236±0.347[b]	0.1620±0.01310[b]	32.09±2.270	6	34.091±0.884	6	50.02±1.36
	January	63	4.204±0.154[a]	0.17359±0.00578[ab]	30.296±0.896	55	33.719±0.930	47	50.04±1.46
	February	73	4.171±0.145[a]	0.18818±0.00547[a]	29.083±0.892	65	35.340±2.370	53	47.03±3.69
Dam age (month)			‡						
	≤24	12	3.578±0.291	0.1798±0.01100	31.51±1.600	11	34.06±1.60	10	46.15±2.51
	>24 and ≤ 36	34	4.175±0.184	0.17766±0.00693	30.47±1.030	30	34.45±1.08	27	49.72±1.70
	>36	97	3.857±0.168	0.16635±0.00634	29.48±1.070	85	34.63±1.08	69	51.22±1.65
Weaning age (day)									
	≤130	46	-	-	29.690±1.350	44	34.200±1.390	36	48.24±2.21
	>130 and ≤145	51	-	-	30.390±1.160	44	34.940±1.190	38	48.64±1.92
	>145 and ≤180	46	-	-	31.386±0.959	38	34.001±0.986	32	50.21±1.54
Birth weight (kg)					***		‡		‡
	≤4.5	93	-	-	28.886±0.892[b]	81	32.847±0.900	69	47.78±1.36
	>4.5	50	-	-	32.090±1.150[a]	45	35.920±1.170	37	50.29±1.90

‡: P<0.10; *:P<0.05; **:P<0.01; ***:P<0.001.
[abc]: Least squares means with different superscript in each fixed effect are significantly different (P<0.05).

Table 2. Least squares means for growth traits of Ramlıç, BC_1 and Texel lambs born in 2016 – 2017.

Factor		n	Birth weight (kg)	Average daily weight gain (kg)	Weaning weight (kg)	n	Weight at six mo. of age (kg)	n	Weight at twelve mo. of age (kg)
	μ	224	4.7943±0.0877	0.20528±0.00431	32.274±0.616	121	35.933±0.876	109	49.37±1.19
Genotype				**	**		***		***
	Ramlıç	98	4.647±0.108	0.21151±0.00530^{a}	33.359±0.735^{a}	53	38.73±1.09^{a}	53	54.86±1.41^{a}
	Ramlıç BC_1 (-/-)	48	4.859±0.136	0.21019±0.00667^{a}	32.569±0.941^{ab}	-	-	-	-
	Ramlıç BC_1 (+/-)	40	4.962±0.135	0.21301±0.00664^{a}	33.468±0.937^{a}	40	36.91±1.04^{a}	34	51.40±1.44^{a}
	Texel (+/+)	38	4.708±0.156	0.18640±0.00769^{b}	29.700±1.070^{b}	28	32.16±1.41^{b}	22	41.85±1.98^{b}
Sex			***	***	***		***		***
	Female	120	4.5911±0.0994^{b}	0.18969±0.00489^{b}	30.472±0.687^{b}	73	33.44±1.00^{b}	66	44.13±1.36^{b}
	Male	104	4.9980±0.1060^{a}	0.22087±0.00523^{a}	34.077±0.752^{a}	48	38.43±1.05^{a}	43	54.61±1.44^{a}
Birth type			***	***	**		‡		‡
	Multiple	66	4.4040±0.1290^{b}	0.19332±0.00634^{b}	31.005±0.876^{b}	36	34.75±1.23	32	47.89±1.63
	Single	158	5.1851±0.0798^{a}	0.21723±0.00392^{a}	33.543±0.602^{a}	85	37.12±0.86	77	50.85±1.20
Birth month				‡	**				
	December	67	4.8280±0.1240	0.21106±0.00609	33.786±0.903^{a}	39	36.15±1.24	36	48.06±1.66
	January	157	4.7611±0.0859	0.19949±0.00423	30.762±0.751^{b}	82	35.72±1.06	73	50.68±1.45
Dam age (month)			*						
	≤24	65	4.9010±0.1260^{ab}	0.20798±0.00621	32.536±0.873	42	36.230±1.100	40	49.43±1.47
	>24 and ≤ 36	21	4.4750±0.1870^{b}	0.20005±0.00922	32.00±1.260	11	36.260±1.930	9	49.09±2.61
	>36	138	5.0064±0.0732^{a}	0.20781±0.00360	32.283±0.552	68	35.306±0.770	60	49.59±1.08
Weaning age (day)					***				
	≤130	65	-	-	29.430±0.960^{b}	34	33.750±1.330	29	46.74±1.87
	>130 and ≤145	123	-	-	33.209±0.718^{a}	67	36.056±0.980	60	48.48±1.30
	>145 and ≤180	36	-	-	34.180±1.180^{a}	20	38.000±1.650	20	52.88±2.17
Birth weight (kg)					***		***		*
	≤4.5	63	-	-	29.824±0.818^{b}	35	33.490±1.170^{b}	29	47.22±1.64^{b}
	>4.5	161	-	-	34.725±0.671^{a}	86	38.376±0.916^{a}	80	51.52±1.21^{a}

‡: $P<0.10$; *:$P<0.05$; **:$P<0.01$; ***:$P<0.001$.
^{abc}: Least squares means with different superscript in each fixed effect are significantly different ($P<0.05$).

Table 3. Least squares means for body and real-time ultrasonographic measurements at weaning in F_1 lambs born in 2015 - 2016.

Factor		n	Wither height (cm)	Body length (cm)	Rump width (cm)	Hearth girth (cm)	MLD depth (cm)	MLD area (cm^2)	Backfat thickness (cm)
	μ	51	58.917±0.831	59.797±0.800	18.530±0.424	80.91±1.99	2.4788±0.0829	9.601±0.451	0.5467±0.0268
Sex								*	
	Female	29	58.617±0.940	59.306±0.905	18.763±0.479	81.72±2.25	2.5202±0.0937	10.192±0.510^{a}	0.556±0.0303
	Male	22	59.218±0.947	60.288±0.912	18.297±0.483	80.11±2.27	2.4375±0.0944	9.010±0.514^{b}	0.5372±0.0306
Birth type			*		‡	‡	‡		
	Multiple	9	57.490±1.330^{b}	59.14±1.28	17.890±0.680	78.19±3.20	2.372±0.133	9.089±0.724	0.5291±0.0431
	Single	42	60.341±0.618^{a}	60.453±0.595	19.170±0.315	83.64±1.48	2.5854±0.062	10.114±0.335	0.5644±0.0199
Birth month			‡	*	‡				
	December	5	64.40±2.48	65.87±2.39^{a}	20.910±1.270	90.05±5.96	2.549±0.248	10.22±1.35	0.6203±0.0802
	January	35	57.05±1.21	58.14±1.16^{ab}	17.940±0.617	78.52±2.90	2.449±0.121	9.548±0.656	0.5147±0.0390
	February	11	55.31±1.80	55.38±1.73^{b}	16.738±0.919	74.17±4.32	2.438±0.180	9.038±0.978	0.5052±0.0582
Dam age (month)									
	≤24	12	59.110±1.250	60.75±1.210	18.863±0.639	81.60±3.00	2.4650±0.1250	10.025±0.680	0.5464±0.0405
	>24 and ≤ 36	17	59.770±1.140	60.07±1.100	18.137±0.581	81.64±2.73	2.5500±0.1130	9.566±0.618	0.5485±0.0368
	>36	22	57.878±0.836	58.567±0.805	18.590±0.426	79.50±2.00	2.4207±0.0833	9.213±0.453	0.5453±0.0270
Birth weight (kg)								‡	
	≤4.5	26	58.672±0.891	59.131±0.858	18.242±0.454	79.91±2.14	2.4254±0.0888	9.100±0.483	0.5360±0.0288
	>4.5	25	59.160±1.030	60.463±0.990	18.818±0.525	81.92±2.47	2.5320±0.1030	10.102±0.558	0.5575±0.0332
Weaning age (day)									
	≤130	23	61.21±1.31	61.33±1.26	19.476±0.666	84.36±3.13	2.459±0.130	9.538±0.709	0.5587±0.0422
	>130 and ≤145	22	60.51±1.42	60.67±1.36	19.170±0.723	84.10±3.40	2.381±0.141	8.995±0.769	0.5852±0.0458
	>145 and ≤180	6	55.03±2.40	57.39±2.31	16.940±1.220	74.28±5.75	2.596±0.239	10.270±1.30	0.4963±0.0775

‡: $P<0.10$; *:$P<0.05$; **:$P<0.01$; ***:$P<0.001$.
^{abc}: Least squares means with different superscript in each fixed effect are significantly different ($P<0.05$).

Table 4. Least squares means for body and real-time ultrasonographic measurements at weaning in BC$_1$ lambs born in 2016 – 2017.

Factor	n	Wither height (cm)	Body length (cm)	Rump width (cm)	Hearth girth (cm)	MLD depth (cm)	MLD area (cm^2)	Backfat thickness (cm)
μ	80	63.080±0.609	60.903±0.704	21.604±0.289	84.27±1.08	3.461±0.124	16.087±0.911	0.7740±0.0258
Genotype						*	*	
Ramlıç BC$_1$ (+/-)	37	62.714±0.686	60.930±0.793	21.633±0.326	83.89±1.22	3.618±0.140^{a}	17.16±1.03^{a}	0.7589±0.0290
Ramlıç BC$_1$ (-/-)	43	63.445±0.684	60.876±0.791	21.574±0.325	84.64±1.21	3.304±0.139^{b}	15.01±1.02^{b}	0.7890±0.0289
Sex		*						
Female	39	62.320±0.680^{b}	60.325±0.786	21.423±0.323	83.92±1.21	3.482±0.138	16.34±1.02	0.7739±0.0287
Male	41	63.839±0.698^{a}	61.480±0.806	21.784±0.331	84.61±1.24	3.440±0.142	15.83±1.04	0.7741±0.0295
Birth type					*			
Multiple	24	63.086±0.837	60.405±0.968	21.708±0.398	82.55±1.49^{b}	3.326±0.170	15.26±1.25	0.7559±0.0354
Single	56	63.073±0.568	61.401±0.657	21.499±0.270	85.98±1.01^{a}	3.596±0.116	16.908±0.851	0.7920±0.0240
Birth month			‡				**	
December	34	63.597±0.768	61.737±0.888	21.784±0.365	84.40±1.36	3.498±0.156	17.63±1.15^{a}	0.7958±0.0325
January	46	62.562±0.692	60.069±0.800	21.423±0.329	84.13±1.23	3.424±0.141	14.54±1.04^{b}	0.7521±0.0292
Dam age (month)		‡						
≤24	8	64.23±1.110	61.44±1.28	21.933±0.528	83.60±1.97	3.335±0.226	15.91±1.66	0.7687±0.0469
>24 and ≤ 36	14	63.058±0.875	60.790±1.01	21.718±0.416	85.18±1.55	3.563±0.178	16.04±1.31	0.8113±0.0370
>36	58	61.951±0.460	60.478±0.532	21.160±0.219	84.021±0.817	3.4849±0.0937	16.305±0.689	0.7419±0.0194
Birth weight (kg)		***	*	**	*	*	**	
≤4.5	21	61.436±0.771^{b}	59.883±0.892^{b}	21.059±0.367^{b}	82.67±1.37^{b}	3.315±0.157^{b}	14.29±1.15^{b}	0.7684±0.0326
>4.5	59	64.723±0.624^{a}	61.922±0.722^{a}	22.148±0.297^{a}	85.86±1.11^{a}	3.607±0.127^{a}	17.881±0.934^{a}	0.7796±0.0264
Weaning age (day)				*	‡			
≤130	8	63.00±1.150	60.85±1.33	20.694±0.548^{b}	81.83±2.05	3.578±0.235	15.06±1.73	0.8065±0.0488
>130 and ≤145	54	62.571±0.541	60.750±0.625	21.569±0.257^{ab}	83.576±0.960	3.307±0.110	16.775±0.810	0.7601±0.0229
>145 and ≤180	18	63.665±0.932	61.110±1.08	22.548±0.443^{a}	87.40±1.650	3.498±0.190	16.430±1.39	0.7553±0.0394

‡: P<0.10; *:P<0.05; **:P<0.01; ***:P<0.001.
abc: Least squares means with different superscript in each fixed effect are significantly different (P<0.05).

Findings showed that birth weight was significantly (P<0.05) affected by genotype, sex, birth type and birth month. The birth weight was found to be highest in F$_1$ lambs whereas Texel lambs were lowest in this trait. The effect of genotype, birth type and birth month on average daily live weight gains were statistically significant (P<0.05) and, Ramlıç and F$_1$ lambs were found to be superior to Texel. Weaning weight was also significantly (P<0.05) affected by genotype, birth type and birth weight. Weaning weight of Ramlıç lambs had higher than the others, while F1 genotype was between the Ramlıç and Texel. The effects of genotype and sex on weights at the six and 12 months of age were also found to be significant (P<0.01). Ramlıç lambs had the highest values in both traits, followed by F$_1$ and Texel. The effects of birth type on wither height, sex on MLD area and, birth month on body length at weaning were significant (P<0.05) in F$_1$ lambs.

Analysis of variance showed that sex, birth type and dam age had a significant (P<0.05) effect on birth weights of all genotypes. The effect of genotypes on birth weight was not significant, but the perusal of the least-squares means revealed that BC$_1$ lambs carrying the mutation tend with the highest birth weight. Daily live weight gains significantly (P<0.01) affected by genotype, sex and birth type. The impact of genotype, sex, birth type, birth month, weaning age and birth weight on weaning weight were statistically significant (P<0.05). Live weights at six and 12 months of age significantly (P<0.05) affected by genotype, sex and birth weight. Differences between Ramlıç and BC$_1$ genotypes were not significant in these traits. The influences of sex on wither height, weaning age on rump width, birth type on chest circumference, genotype on MLD depth and genotype and birth month on MLD area were found to be statistically significant (P<0.05) in BC$_1$ lambs. The birth weight was also found to be significant (P<0.05) in all traits, except MLD depth (P<0.10) and backfat thickness.

Discussion and Conclusion

There was no significant difference in birth weight between pure Ramlıç and F$_1$ lambs in first lambing season and BC$_1$ lambs in second lambing season. The least-squares means showed that crossbreds carrying myostatin mutation tended to be heavier in birth. Similar findings were reported in Romneys (17) and Iranian Makoei sheep

(14). The birth weight values determined for BC_1 lambs are just above the range of 4.00 to 4.95 kg reported for Ramlıç and Rambouillet in the literature (6, 7, 12, 15, 18, 21, 30, 34, 35). The birth weight values in F_1 and Texel lambs are consonant with the findings (3.7 - 5.1 kg) of literature (3, 13, 22, 23, 26, 33) for lambs carrying different level of Texel genotype. Sex, type of birth and month of birth had a significant effect ($P<0.05$) on birth weight in consistent with the results of various researchers (11, 15, 34, 35). The daily live weight gain of Ramlıç, F_1 and BC_1 lambs were found to be convenient with the literature (1, 6, 18, 19, 30) reported for Ramlıç, Rambouillet and its crossbreeds. Average daily live weight gain of Texel lambs were slightly short of the 0.190 - 0.318 kg limits reported in other studies (2, 10, 26). The differences may be due to the variations in feeding and management, weaning times, and statistical models used in various researches. The weaning weights of Ramlıç, F_1 and BC_1 lambs determined in this study were found to be higher than the values amongst 24.1 and 28.6 kg reported in previous literature (11, 12, 15, 34, 35). Likewise, to Ramlıç and its crosses of our study, Bromley et al. (6) notified that the weaning weights for Columbia, Polypay, Rambouillet and Targhee lambs as 36.4 kg, 33.7 kg, 32.7 kg and 33.8 kg, respectively. The weaning weights of Texel lambs in this study were in the range of 20.9 – 31.2 kg reported by McEwan et al. (27) and McMillan et al. (28) for purebred and crossbred Texel lambs. It was also found just above 26.8 kg that was obtained by Wuliji et al. (33) in Texel x Romney crossbred and below the value of 34.42 kg reported by Khusro et al. (22). The weight findings at six months of age for Ramlıç and BC_1 lambs were higher than that of Yalçın et al. (34) and Ceyhan et al. (7) for Ramlıç and Rambouillet. The MLD depth of F_1 lambs were lower than the British Texel lambs, while higher in BC_1 lambs (32). The significant difference between myostatin carriers and non - carriers for MLD depth and MLD area may have been derived from the effect of myostatin mutation. The body length of F_1 and BC_1 lambs and chest girth in BC_1 lambs were also higher than the results of the same researchers. This situation suggested that repeated backcrossing has no harmful effect on body size.

It was determined that the factors such as genotype, sex, type of birth, maternal age, weaning age and birth weight may have significant effects on the pre- and post-weaning growth characteristics of lambs. These factors should be taken into consideration in selection and backcrossing studies in some special situations such as detecting of myostatin mutation. The trend in least-squares means for weaning weight showed that the myostatin heterozygous BC_1 lambs had been potentially heavier than the non-carriers and pure Texel. Their weights at six and twelve months of age were not statistically significant from Ramlıç lambs. MLD depth and area were significantly higher ($P<0.05$) in BC_1 lambs carrying myostatin than non – carriers. Consequently, findings revealed that the beginning phase of introgression processes practiced in this study has been succeeded.

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Ethical Statement

This study was approved by the animal ethics committee of the Afyon Kocatepe University (AKUHADYEK) Decision no.49533702-26.

Conflict of interest

All authors declare there are no conflicts of interest among them and with any other people or corporations.

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