VARIATION IN INTRON 1 OF THE OVINE GDF8 GENE AND ITS ASSOCIATION WITH GROWTH AND CARCASS CHARACTERISTICS OF DUAL PURPOSE SHEEP

A. H. M. Ibrahim*[1], H. Zhou[2] and J.G.H. Hickford[2]

1- Department of Animal Breeding, Desert Research Center, 1 Mathaf AlMatariya St., Cairo, Egypt, 2- Gene-Marker Laboratory, Agriculture and Life Sciences Division, P.O. Box 84, Lincoln University, Canterbury, New Zealand
**** Email: adelhosseiny2005@yahoo.com***

SUMMARY

The aim of this study was to further investigate the variation in growth and differentiation factor 8 (GDF8) gene and its association with growth and carcass characteristics of New Zealand Romney sheep. Polymerase chain reaction-single strand conformational polymorphism (PCR-SSCP) analysis was used to detect the polymorphisms in intron 1 of ovine GDF8 gene in 447 New Zealand Romney lambs produced from 17 sire-lines. PCR-SSCP analysis identified a total of six SSCP genotypes, AA (0.111), AB (0.367), AC (0.100), BB (0.288), BC (0.128) and CC (0.006) representing three alleles A, B and C with frequency of 0.34, 0.54 and 0.12, respectively. General linear mixed effect models revealed a significant effect (P< 0.05) for GDF8 genotype on loin yield and percentage loin yield. The presence of allele B was significantly associated with increased loin yield (P< 0.05) and percentage loin yield (P< 0.01). Effects of number of allele copies present on the studied traits were assessed. Loin yield and percentage loin yield were significantly (P< 0.05) affected by number of allele B copies. Having one copy of allele B was associated with increased loin yield, total yield and percentage loin yield; however, having two copies of allele B was associated with decreased loin yield and total yield. Our present results suggest that GDF8 polymorphism is mainly associated with loin yield and percentage loin yield and has no effect on birth weight, weaning weight and growth rate in New Zealand Romney sheep.

Keywords: growth, carcass, GDF8, PCR-SSCP, Romney sheep

INTRODUCTION

The identification of genes that affect economically important traits for sheep meat would improve selective breeding programs for sheep production. Growth and differentiation factor 8 (GDF8) is one such gene. It is a member of the transforming growth factor β (GDF8 β) superfamily. The members of this family regulate cell growth and differentiation in both embryonic and adult tissues. GDF8 is highly expressed in developing and adult muscles and acts as a negative regulatory factor by inhibiting MYF5 and MyoD two factors involved in the differentiation of muscle precursor cells into myoblasts (McPherron *et al*., 1997).

GDF8 loss-off function leads to increase skeletal muscle mass (double muscling) in mice (McPherron *et al*., 1997; Szabo *et al*., 1998; Lin *et al*., 2002; Whittemore *et al*., 2003 and Mendias *et al*., 2008). While variation in other species including cattle (McPherron and Lee, 1997; Grobet *et al*., 1997; Kambadur *et al*., 1997; Wiener *et al*., 2002; Marchitelli *et al*., 2003 and Grisolia *et al*., 2009), Sheep (Clop *et al*., 2006; Kijas *et al*., 2007; Hickford *et al*., 2009; Han *et al*., 2010 and Haynes *et al*., 2013) and human (Schuelke *et al*., 2004) is reported to affect muscling. In addition, GDF8 deficiency in mice reduces adipogensis (Lin *et al*., 2002; McPherron and Lee, 2002), as a result of reduced production and secretion of leptin (McPherron and Lee, 2002). The effect of GDF8 on myogenic and adipogenic differentiation potentially has important implications for growth and carcass traits of sheep. The ovine GDF8 gene consists of three exons and two introns (Bellinge *et al*., 2005) and located on chromosome 2.

Variation in the ovine GDF8 gene and its effect on important production traits in sheep has been described in a number of reports. For example a single nucleotide polymorphism (SNP; g.6223G>A) has been detected in the 3'- UTR of GDF8 gene in Belgian Texel sheep (Clop *et al*., 2006). The same SNP was also detected in other breeds including Australian Texel sheep (Kijas *et al*., 2007), Charollais sheep (Hadjipavlou *et al*., 2008), New Zealand Texel sheep (Johnson *et al*., 2009) and White Suffolk, Poll Dorest and Lincoln sheep in Australia (Kijas *et al*., 2007). This SNP has been found to affect muscle hypertrophy in Belgian Texel sheep (Clop *et al*., 2006), muscle depth in Charollais sheep (Hadjipavlou *et al*., 2008) and birth weight, mean lean yield and total muscle yield in New

Zealand Romney sheep (Han *et al*., 2010). Additional SNPs (g^- 41C>A, g^+ 4036A>C and g^+ 6223G>A) have been identified in the promoter and intron 2 regions and showed significant effects on slaughter measurements of muscling and fatness (Kijas *et al*., 2007). Additional two SNPs (C.2360G>A and C.960delG) have been detected and are reported to reduce fatness and increase muscle mass in Norwegian White sheep (Boman *et al*., 2010). A single strand conformational polymorphism analysis (SSCP) of the 473-bp of the exon 1- intron 1 region of GDF8 gene has revealed three allelic variants in NZ Romney sheep (Zhou *et al*., 2008). In the same breed, five SSCP allelic variants have been detected in intron 1 region of GDF8 gene and showed significant effects on leg yield, loin yield, loin yield % and total yield (Hickford *et al*., 2009).

The objective of the present study was to further investigate allelic variants of intron 1 of the ovine GDF8 gene and to test their association with growth and carcass traits in New Zealand Romney sheep.

MATERIALS AND METHODS

Animals and data collection

A total of 447 male Romney lambs produced by 17 unrelated NZ Romney rams were used to detect the allelic variation in intron 1 of GDF8 and then to test the association of the variants with growth and carcass traits for Romney sheep.

Birth date, birth rank (i.e. whether they were single, twin or triple) and gender were recorded at birth. Subsequently, the growth data were collected including birth weight, weaning weight, weaning age, pre-weaning growth rate (calculated as the difference between weaning weight and birth weight divided by age in days; expressed in grams/day), and weight and age at selection (on farm) for slaughter.

Hot carcass weight (HCW) was measured directly on the processing chain. HCW is the weight in kilograms of the carcass components minus the pelt, head and gut. Video imaging analysis (VIASCAN®Sastek), developed by Meat and Livestock Australia and described by Hopkins *et al*. (2004), was used to estimate the following carcass traits: lean meat yield (expressed as a percentage of HCW) in the leg (leg yield), loin (loin yield) and shoulder (shoulder yield), total yield (the sum of the leg, loin and shoulder yields for any given carcass), the proportion leg yield, the proportion loin yield and the proportion shoulder yield. The proportion yield of leg, loin or shoulder is the yield of the specific area, divided by the total yield expressed as a percentage.

DNA purification and genotyping

Blood samples were collected on FTA cards. For each sample, a disc of 1.2 mm in diameter was punched and the genomic DNA was purified from the dried blood spot using a two-step procedure described by Zhou *et al*. (2006).

A 414 bp fragment containing intron 1 of GDF8 gene was amplified using a pair of specific primers. The sequences of these two primers are described in the report of Hickford *et al*. (2009) and are as follows: F: 5'-GAAACGGTCATTACCA-TGC-3' and R: 5'-CAT-ATTTCAGGCAACCAAATG-3'. PCR amplification was carried out in a total reaction volume of 20 µl containing the genomic DNA on the FTA card, 0.25 µM of each primer, 150 µM of Mg^{++}, 0.5 U of Taq DNA polymerase and 1x reaction buffer supplied. The reaction conditions were as follow: an initial DNA template denaturation at 94 C° for 2 min, followed by 35 cycles of denaturation at 94 C° for 30 sec; annealing at 61 C° for 30 sec, and extension at 72 C° for 40 sec and final extension at 72 C° for 5 min.

One µl of each amplicon was mixed with 10 µl of loading dye (98% formamide, 10 mM EDTA, 0.025 % bromophenol blue, 0.025% xylene cyanol). After denaturation at 105 C° for 5 min, samples were rapidly cooled on wet ice and then loaded on 12% acrylamide gels. Amplicons representive of the three known GDF8 alleles (Hickford *et al*., 2009) were also included in each polyacrylamide gel to use their banding patterns as a standard for determining the alleles present in individual lambs. Electrophoresis was performed using Protean IIxi cells (Bio-Rad), at 350V and 12 C° for 18 h in 0.5x TBE buffer. Gels were silver-stained according to the method described by Byun *et al*. (2009).

Statistical Analysis

Data were analyzed using SPSS version 15 (SPSS Science Inc., Chicago, IL). The strength of the associations between the various traits was tested by calculating Pearson correlation coefficients. No traits were strongly correlated, so they were all tested independently.

General linear mixed effect models (GLMMs) were used to assess the effect of GDF8 genotypes (frequency < 1%) on growth and carcass traits. GDF8 genotype and birth rank were fitted as fixed factors along with sire fitted as a random factor in each model. In the model assessing the genotype effect on weaning weight, weaning age was included as a covariate. Also, draft weight was included as a covariate in the model testing the effect of genotypes on draft age and HCW.

The generalized statistical model used to test the genotype effect was (without the added covariates) as follows:

$$\boldsymbol{Y_{ijkl} = \mu + t_i + \beta_j + \alpha_k + \epsilon_{ijkl}}$$

Where $\boldsymbol{Y_{ijkl}}$ = traits (birth weight, weaning weight, growth rate, etc); $\boldsymbol{\mu}$ = the overall mean for each trait; $\boldsymbol{t_i}$ = the fixed effect of i^{th} genotype; $\boldsymbol{\beta_j}$ = the fixed effect of j^{th} birth rank; $\boldsymbol{\alpha_k}$ = the random effect of k^{th} sire and $\boldsymbol{\epsilon_{ijkl}}$ = the random error for *ijkl*.

The GLMMs were used to explore the effect of the absence/presence of myostatin alleles on growth and carcass traits. For each trait, a GLMM was performed for each GDF8 allele observed in the population. Allele absence or presence (coded as 0 or 1, respectively), and birth rank were fitted as fixed factors, whilst sire was fitted as a random factor in

each model. Co-variates were fitted as described above.

A separate set of analyses was performed to test the effect of the number of allele copies present on growth and carcass traits. The GLMMs were conducted in an identical manner to the models used for testing the absence/presence of each allele. Similar to the absence/presence models, each allele was tested in separate models. Co-variates were fitted as described above.

Where significant ($P \leq 0.05$) or if tending towards significance ($0.05 < P \leq 0.1$), these were further explored using pairwise comparisons (least significant difference).

RESULTS AND DISCUSSION

PCR-SSCP analysis of intron 1 of the GDF8 gene in New Zealand Romney sheep revealed only three alleles (A, B and C) with frequency of 0.34, 0.54 and 0.12, respectively. Six genotypes were observed in the genotyped lambs including AA (0.111), AB (0.367), AC (0.100), BB (0.288), BC (0.128) and CC (0.006). Fifteen SSCP genotypes representing five alleles (A, B, C, D and E) were detected in a variety of breeds and composite breeds in New Zealand. Variation in the same region of New Zealand Romney sheep has been also described by Hickford *et al.* (2009). They also reported six GDF8 genotypes AA (0.466), AB (0.302), AC (0.133), BB (0.058), BC (0.035) and CC (0.06), derived from three alleles A (0.683), B (0.227) and C (0.09).

PCR-SSCP analysis proved to be a robust tool to detect the mutation in GDF8 gene in sheep using gene sequence information to construct the primers and also could be used to screen a large number of samples because of its low price, speedy and easy handling.

To assess the effect of GDF8 genotype on growth and carcass traits, only the common genotypes AA, AB. AC, BB and BC were included, as the frequency of CC was less than 1%. No associations were found between GDF8 genotypes and birth weight, weaning weight, growth rate or draft-age. The GLMM results (Table 1) suggested a significant effect ($P < 0.05$) for the genotype on loin yield and percentage loin yield. In addition, GLMMs suggested an association, although not significant ($P < 0.095$), between total yield and genotype. Least square mean results showed that lambs with the genotype BC had the highest mean and lambs with the genotype AC had the lowest mean for loin yield and total yield. Also, the genotype BC had the highest mean and the genotype AA had the lowest mean for percentage loin yield. Pairwise comparison results indicated that the lambs with the genotype BC were higher ($P < 0.05$) than the lambs with the genotype AA. These results are partially consistent with the observations made by Hickford *et al*. (2009) who found that GDF8 genotypes significantly affected leg yield, loin yield, total yield and the percentage loin yield.

Table 1. The effect of GDF8 genotype on various assessments of yield

Trait	LSM ± SE					P-value
	AA (n= 49)	AB (n= 171)	AC (n= 47)	BB (n= 139)	BC (n= 63)	
Leg Yield (Kg)	21.810±0.160	21.696±0.093	21.456±0.158	21.497 ± 0.100	21.678 ± 0.144	0.214
Loin Yield (Kg)	14.675±0.124	14.810±0.072	14.592±0.123	14.631 ± 0.078	14.977 ± 0.112	0.024
Shoulder Yield (Kg)	17.544±0.125	17.532±0.072	17.336±0.123	17.407 ± 0.078	17.528 ± 0.112	0.425
Total Yield (Kg)	54.030±0.342	54.039±0.199	53.385±0.338	53.535 ± 0.213	54.182 ± 0.308	0.095
Leg Yield %	0.403±0.001	0.401±0.001	0.402±0.001	0.401 ± 0.001	0.400 ± 0.001	0.436
Loin Yield %	0.272±0.001	0.274±0.001	0.273±0.001	0.273 ± 0.001	0.277 ± 0.001	0.022
Shoulder Yield %	0.325±0.002	0.325±0.001	0.325±0.002	0.325 ± 0.001	0.323 ± 0.001	0.790

Table 2. Association of GDF8 alleles with various assessments of yield

Trait	Allele being assessed	Other alleles in model	LSM ± SE				P-value
			Allele absent	N	Allele present	N	
Leg yield (Kg)	A	None	21.567 ± 0.088	204	21.672 ± 0.081	267	0.267
	B	None	21.646 ± 0.117	98	21.621 ± 0.731	373	0.834
	C	None	21.635 ± 0.074	359	21.596 ± 0.115	112	0.743
	A	B, C	21.547 ± 0.110	204	21.663 ± 0.089	267	0.293
	B	A, C	21.588 ± 0.129	98	21.622 ± 0.086	373	0.811
	C	A, B	21.611 ± 0.096	359	21.599 ± 0.115	112	0.932
Loin yield (Kg)	A	None	14.743 ± 0.069	204	14.748 ± 0.063	267	0.945
	B	None	14.644 ± 0.092	98	14.771 ± 0.057	373	0.166
	C	None	14.725 ± 0.058	359	14.820 ± 0.090	112	0.303
	A	B, C	14.660 ± 0.085	204	14.771 ± 0.070	267	0.200
	B	A, C	14.591 ± 0.101	98	14.841 ± 0.067	373	0.026
	C	A, B	14.620 ± 0.075	359	14.812 ± 0.090	112	0.059
Shoulder yield (Kg)	A	None	17.450 ± 0.068	204	17.497 ± 0.063	267	0.518
	B	None	17.445 ± 0.091	98	17.484 ± 0.057	373	0.669
	C	None	17.484 ± 0.058	359	17.450 ± 0.089	112	0.708
	A	B, C	17.409 ± 0.085	204	17.486 ± 0.070	267	0.374
	B	A, C	17.407 ± 0.101	98	17.488 ± 0.067	373	0.469
	C	A, B	17.446 ± 0.075	359	17.449 ± 0.089	112	0.975
Total yield (Kg)	A	None	53.759 ± 0.188	204	53.919 ± 0.173	267	0.436
	B	None	53.735 ± 0.251	98	53.876 ± 0.156	373	0.575
	C	None	53.844 ± 0.159	359	53.866 ± 0.246	112	0.932
	A	B, C	53.616 ± 0.235	204	53.921 ± 0.191	267	0.200
	B	A, C	53.587 ± 0.277	98	53.951 ± 0.185	373	0.237
	C	A, B	53.677 ± 0.205	359	53.681 ± 0.246	112	0.510
Leg yield%	A	None	0.401 ± 0.001	204	0.402 ± 0.001	267	0.299
	B	None	0.403 ± 0.001	98	0.401 ± 0.001	373	0.173
	C	None	0.402 ± 0.001	359	0.401 ± 0.001	112	0.406
	A	B, C	0.402 ± 0.001	204	0.402 ± 0.001	267	0.926
	B	A, C	0.402 ± 0.001	98	0.401 ± 0.001	373	0.147
	C	A, B	0.402 ± 0.001	359	0.401 ± 0.001	112	0.205
Loin yield %	A	None	0.274 ± 0.001	204	0.274 ± 0.001	267	0.342
	B	None	0.272 ± 0.001	98	0.274 ± 0.001	373	0.060
	C	None	0.273 ± 0.001	359	0.275 ± 0.001	112	0.084
	A	B, C	0.273 ± 0.001	204	0.274 ± 0.001	267	0.527
	B	A, C	0.272 ± 0.001	98	0.275 ± 0.001	373	0.010
	C	A, B	0.272 ± 0.001	359	0.275 ± 0.001	112	0.010
Shoulder yield%	A	None	0.325 ± 0.001	204	0.325 ± 0.001	267	0.952
	B	None	0.325 ± 0.001	98	0.325 ± 0.001	373	0.872
	C	None	0.325 ± 0.001	359	0.324 ± 0.001	112	0.353
	A	B, C	0.325 ± 0.001	204	0.324 ± 0.001	267	0.735
	B	A, C	0.325 ± 0.001	98	0.324 ± 0.001	373	0.550
	C	A, B	0.325 ± 0.001	359	0.324 ± 0.001	112	0.275

Table 3. Association of GDF8 allele copy number with various assessments of yield

Trait	Allele being assessed	LSM ± SE						P-value
		Allele absent	N	Allele 1 copy	N	Allele 2 copy	N	
Leg yield (Kg)	A	21.566± 0.088	204	21.646± 0.085	218	21.813± 0.120	49	0.321
	B	21.648± 0.117	98	21.693± 0.084	234	21.505± 0.099	139	0.226
	C	21.632± 0.074	359	21.586 ± 0.115	110	22.211± 0.708	2	0.644
Loin yield (Kg)	A	14.744± 0.069	204	14.762 ± 0.067	218	14.677± 0.215	49	0.801
	B	14.646± 0.091	98	14.854 ± 0.065	234	14.635± 0.077	139	0.015
	C	14.723± 0.058	359	14.811 ± 0.090	110	15.357± 0.553	2	0.363
Shoulder yield (Kg)	A	17.449± 0.068	204	17.489 ± 0.066	218	17.544± 0.124	49	0.740
	B	17.446± 0.091	98	17.530 ± 0.065	224	17.409± 0.077	139	0.331
	C	17.483± 0.058	359	17.445 ± 0.090	110	17.756± 0.551	2	0.795
Total yield (Kg)	A	53.759± 0.188	204	53.897 ± 0.182	218	54.033± 0.343	49	0.685
	B	53.741± 0.250	98	54.077 ± 0.179	234	53.550± 0.212	139	0.068
	C	53.839± 0.159	359	53.841 ± 0.247	110	55.324± 1.518	2	0.621
Leg yield%	A	0.401 ± 0.001	204	0.401 ± 0.001	218	0.403 ± 0.001	49	0.231
	B	0.403 ± 0.001	98	0.401 ± 0.001	234	0.401 ± 0.001	139	0.384
	C	0.402 ± 0.001	359	0.401 ± 0.001	110	0.401 ± 0.001	2	0.708
Loin yield%	A	0.274 ± 0.001	204	0.274 ± 0.001	218	0.272 ± 0.001	49	0.148
	B	0.272 ± 0.001	98	0.275 ± 0.001	234	0.273 ± 0.001	139	0.037
	C	0.273 ± 0.001	359	0.275 ± 0.001	110	0.279 ± 0.001	2	0.180
Shoulder yield%	A	0.325 ± 0.001	204	0.325 ± 0.001	218	0.325 ± 0.002	49	0.972
	B	0.325 ± 0.001	98	0.324 ± 0.001	234	0.325 ± 0.001	139	0.642
	C	0.325 ± 0.001	359	0.324 ± 0.001	110	0.321 ± 0.007	2	0.569

As shown in Table (2), the presence/ absence of particular allele were not found to affect birth weight, weaning weight, growth rate or draft-age. The presence of allele B tended to be associated with the increased percentage loin yield ($P < 0.06$), while the presence of allele C tended to be associated with increased HCW ($P < 0.058$) and increased percentage loin yield ($P < 0.084$). The effect of allele B became significant on loin yield ($P < 0.026$) and the percentage loin yield ($P < 0.010$) when alleles A and C were introduced into the model. Furthermore, the effect of allele C on the percentage loin yield became significant ($P < 0.010$) when alleles A and B were introduced into the model (Table 2). When the alleles B and C were forced into the model, the percentage loin yield became more affected by the absence/presence of allele B (absent: 0.272± 0.001, present: 0.275 ± 0.001; $P < 0.009$) and allele C (absent: 0.273 ± 0.001, present: 0.275 ± 0.001; $P < 0.012$). These results suggest that the selection for the presence of either B or C allele would increase the percentage of loin yield.

The third set of analyses (Table 3) concerned the number of GDF8 allele copies present. The number of allele B copies significantly affected loin yield ($P < 0.015$), and percentage loin yield ($P < 0.037$). Having one copy of allele B was associated with increased loin yield, total yield and percentage loin yield, however having two copies of allele B was associated with decreased loin yield and total yield. In contrast to our results, Hickford *et al*. (2009) reported that, having two copies of allele B increases leg yield, loin yield, total yield, and percentage loin yield.

The GLMMs that were used in the three sets of analyses showed that the variation in GDF8 gene had no effect on birth weight, pre-weaning growth rate, draft age or H-W (data not shown). These results are consistent with the findings of Kijas *et al*. (2007) in Australian White Suffolk, Poll Dorest and Lincoln sheep and Hickford *et al*. (2009) in New Zealand Romney sheep. However, the genetic variation was found in intronic DNA, which makes it difficult to explain how the variation affected the activity of GDF8. Possibilities include that the intronic sequence may harbor important functional elements that affect gene expression and RNA splicing (Lomelin *et al*., 2010). It may also be linked to nucleotide variation in critical gene control regions (Hickford *et al*., 2009).

According to our results, the variation in intron 1 of GDF8 gene is correlated with loin yield and percentage loin yield and tends to correlate with the total yield. These traits are the most important carcasses traits of lambs that provide optimal returns

to the farmers, as the loin meat is very tender and is invariably cooked using a dry-heat method and also may be de-boned to produce boneless roasts or chops that consumers prefer. These results could speculate that selection pressure for the correlated traits with this region may have reduced genotypic variation in this breed of sheep.

We could conclude that, although the variation in intron 1 of ovine GDF8 gene affected loin yield and percentage loin yield, further investigations need to be carried out to assess the effect of variation in another regions in this gene on growth and carcass traits of New Zealand Romney sheep and another breeds. Furthermore, our results proved that, PCR-SSCP is an appropriate tool to detect the variability of the candidate genes affecting important traits of farm animals.

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التباين في منطقة الأنترون ١ لجين GDF8 وارتباطه مع صفات النمو والذبيحة في الأغنام ثنائية الغرض

عادل حسيني محمد إبراهيم[١] ، هوتونج زهاو[٢]، جوناثان هيكفورد[٢]

١ - قسم تربية الحيوان، مركز بحوث الصحراء، ١ ش متحف المطرية، القاهرة، جمهورية مصر العربية، ٢ - معمل الأدلة الوراثية، كلية الزراعة وعلوم الحياة، جامعة لنكولن، نيوزيلندا

- تعتبر صفات النمو والذبيحة من أهم الصفات ذات القيمة الاقتصادية في الأغنام ، وهذه الصفات ذات طبيعة كمية حيث يتحكم فيها عدد كبير من الجينات.
- تتم حتى الآن عملية التحسين الوراثي لهذه الصفات باستخدام الطرق التقليدية للانتخاب التي تعتمد على تسجيل القيم المظهرية للصفة المرغوب تحسينها ، ويعاب على هذه الطرق التكلفة العالية والبطء خاصة في الأنواع ذات مدة الجيل الطويلة.
- حديثا قام علماء الوراثة الجزيئية باستباط بعض التقنيات التي يمكن استخدامها في اجراء عملية التحسين الوراثي لحيوانات المزرعة ، ومن أهم هذه التقنيات الانتخاب باستخدام الأدلة الوراثية (Marker assisted selection) ، وباستخدام هذه الطريقة يمكن انتخاب الحيوان بناءا على تركيبة الوراثي فقط و في مرحلة مبكرة من العمر (لذا فان هذه الطريقة أكثر كفاءة وسرعة ودقة مقارنة بالطرق التقليدية).
- وهناك طريقتان لتحديد الدليل الوراثي الانتخابي لصفة ما وهما ، اجراء عملية المسح الجينومي (Genome scan approach) أو دراسة الجين ذات التأثير واسع المدى (Candidate gene approach). و يتم في الطريقة الأولى اجراء مسح جينومي لتحديد كل التتابعات النيوكلوتيدية المرتبطة بالصفة المدروسة ، ويعاب على هذه الطريقة التكلفة العالية جدا ، حيث يجب اجراء عملية المسح الجينومي لعدد كبير من الحيوانات. ويتم في الطريقة الثانية دراسة الأشكال الأليلية والتراكيب الوراثية لجين ما . أثبتت الدراسات الفسيولوجية أن الفعل البيولوجي مرتبط مع القيمة المظهرية للصفة المدروسة، وهل للأشكال الأليلية والتراكيب الوراثية ارتباط مع القيمة المظهرية للصفة، وتتميز هذه الطريقة بالسهولة وقلة التكلفة.
- تم في هذه الدراسة اختيار طريقة الجين واسع المدى لتحديد أدلة وراثية انتخابية لصفات النمو والذبيحة في أغنام الرومني ثنائية الغرض.
- يعتبرالعامل GDF8 من العوامل الهامة جدا التي تؤثر على نمو وانقسام الخلايا في المرحلة الجنينية ومرحلة ما بعد الولادة في كل الأنواع الثديية ، ويتم التشفير لهذا العامل بواسطة جين GDF8 ، وجد أن الطفور في هذا الجين يؤدى الى حدوث زيادة كبيرة في الكتلة العضلية للعديد من الأنواع الثديية مثل الأبقار – الفئران – الانسان. لذا كان من المهم دراسة تأثير التباين في هذا الجين على الصفات المدروسة.
- تم تحديد الأشكال الأليلية والتراكيب الوراثية في منطقة الأنترون ١ لجين GDF8 لعدد ٤٤٧ من حملان أغنام الرومني النيوزيلندية باستخدام تقنية PCR-SSCP .
- تم اجراء عملية التحليل الاحصائي لدراسة تأثير كل من (التركيب الوراثي – وجود الأليل من عدمه في التركيب الوراثي – عدد النسخ لكل أليل في التركيب الوراثي) وذلك على صفات النمو (الوزن عند الميلاد –الوزن عند الفطام – معدل النمو من الميلاد للفطام) والذبيحة (وزن الكتفين – وزن القطن – وزن الفخذين- وزن الذبيحة الكلي- نسبة الكتفين – نسبة القطن- نسبة الفخذين) وذلك في حملان أغنام الرومني.

- وكانت النتائج المتحصل كالتالي

١. تم تحديد عدد ٣ أشكال أليلية لهذا الجين ورمز لها بـ A ، B ، C وكانت تكراراتها(0.34 ، 0.54 ، 0.12) على التوالي ، كما تم تحديد عدد 6 تراكيب وراثية ، هي AA(0.111) ، AB (0.367) ، AC(0.100) ، BB (0.288) ، BC (0.128) ، CC (0.006).

٢. أثبتت نتائج التحليل الاحصائي الآتي:

أ. كان للتركيب الوراثي الخاص بجين GDF8 تأثيرا معنويا ($P < 0.05$) على كل من وزن القطن ونسبة القطن ولم يكن هناك أى تأثير على بقية الصفات.

ب. كان لوجود الأليل B في التركيب الوراثي تأثيرا معنويا على زيادة كل من وزن القطن ($P < 0.05$) ونسبة القطن منسوبة لوزن الذبيحة ($P < 0.01$).

ج. كان لعدد الأليلات الموجودة في التركيب الوراثي تأثيرا معنويا ($P < 0.05$) على كل من وزن القطن ونسبة القطن ، حيث وجد أن وجود عدد ١ نسخة من الأليل B في التركيب الوراثي مرتبطا بزيادة معنوية ($P < 0.05$) لكل من على كل من وزن القطن ونسبة القطن وكذلك الوزن الكلي للذبيحة. بينما وجد أن عدد نسختان من الأليل B ارتبط بحدوث انخفاض في كل من وزن القطن والوزن الكلي للذبيحة

التوصيات:

- وفقا للنتائج المتحصل عليها يوصى في هذه الدراسة بالانتخاب للحملان الحاملة للتركيب الوراثي BC الخاص بالجين GDF8 ، وذلك للحصول على حملان ذات وزن ذبيحة كبير ونسبة قطن أعلى. كما يوصى باجراء دراسات أخرى على التباين في مناطق اخرى من هذا الجين لمعرفة تأثير هذا الجين على صفات النمو والذبيحة في الأغنام.