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SPECIFIC GENES AFFECTING BODY WEIGHT IN IRAQI AWASSI SHEEP

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ABSTRACT

The present study was to investigate genotypes of three genes (MSTN, CAST and MHC-DRB1) that related to body weight in Awassi lambs. A total of 52 lambs were used. The DNA concentration and DNA purity (A260/280) was calculated automatically by nanodrop and ranged between 10.53-53.94 ng/µl and 1.51-2.19, respectively. The RFLP-PCR results showed that the CAST and MHC-DRB1genes were polymorphism among individuals while the MSTN gene was monomorphism. Lambs with CC genotype for MHC-DRB1 locus have positive effect on body weight at most ages while lambs with AB genotype for CAST locus gave higher body weight at all ages and higher average daily gain. The lambs with BBCCBB genotypes for the three genes under study gave higher weight at weaning (22.15 kg/lamb) and higher average daily gain (0.197 kg/lamb/day) compared with all other genotypes. These results showed that Awassi lambs have potential for growth traits and the selection process with the aid of molecular technique can play a positive and rapid role for improvement this breed of sheep.

KEYWORDS: RFLP-PCR, **MSTN, CAST, MHC-DRB1, Weaning weight**.
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INTRODUCTION

The native sheep breeds in Iraq are fat-tailed and carpet-wool type and are raised mainly for lamb and mutton production (Al-Rawi *et al.,* 1996; Al-Azzawi *et al.,* 1995; Al-Barzinji and Gardi, 2012 and 2015). The Awassi sheep, the most numerous small ruminant breed of Iraq (58.2%), are predominantly white with brown or red faces (FAO, 2000). Selection of farm animals depends on the availability of information's in animals. Classic breeding methodology by estimating breeding values requires a lot of efforts, time and fund. Identifying major genes can speed up (at more precise and accurate) genetic improvement of economic traits of sheep breeds. Identification and characterization of breeds is required to identify the genetic resources and also to prioritize breeds for conservation and development. Recently, the use of molecular markers, revealing polymorphism at the DNA level, has been playing an increasing role in animal genetics studies (Baumung *et al.,* 2004).

Improvement of livestock has focused on the selection of breeding individuals with superior phenotypes. With the development of increasingly advanced statistical methods that maximize selection for genetic gain, this simple approach has been extremely successful in increasing the quantity of agricultural output and productivity. However, information now available on the organization and functioning of the genome could be used in breeding programmers to improve a range of traits. Strategies to identify markers for traits and the application of these markers are described by reference to examples of loci that control a range of different traits (Williams, 2005).

Therefore, the objective of the present study is to identify the genetic polymorphism and best genotypes for lamb's growth using PCR-RFLP for three specific genes MSTN, CAST and MHC-DRB1.

MATERIALS AND METHODS

Experimental Animals and Locations

This study was undertaken in commercial flock of Awassi sheep at the Bashblakh village / Kirkuk governorate (30 km east of Kirkuk). Ewes are exposed to rams for mating during end of May to the end of October, so that ewes

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lambing occurred by end of October to end of November. A total of 52 ewes lambed out of 58 ewes mated, giving 52 lambs, six of them died during experiment. At lambing ewes and lambs were identified. Sexes, month of lambing were recorded. Ewes and lambs were raised together until weaning at 90 days of age. Male and female lambs were weighed at birth, one, two and three months of age.

Blood Collection and DNA Extraction

Whole blood was collected from each lamb at the end of the experiment through jugular vein into 5 ml Vacutainer tubes containing the anticoagulant, ethylenediaminetetra-acetic acid (EDTA). Samples were stored at -20°C until DNA extraction. All research works were carried out in both laboratory of Animal Molecular Genetics/College of Agriculture and Molecular Biology/Scientific Research Center/ Salahaddin University. Genomic DNA was isolated from samples using extraction kit (GeNet Bio, koria). The quantity and quality of DNA was checked by Nanodrop spectrophotometer and gel electrophoresis.

PCR Primers

The PCR programs were occurred out according to RFLP strategy portrayed by Davis *et al.*, (2000), Hanrahan *et al.,* (2004), Chu *et al.,* (2007) and Moradband *et al.,* (2011). Primers name, sequencing of primers with their restriction fragments are recorded in tables (1 and 2).

Table (1): Primers, Restriction Enzymes and restriction site for Candidate genes.

Gene symbol	Primer sequences		RE	Restriction site	Reference
	Forward	Reverse			
CAST	5'-TGGGGC CCAATGACGCCATG ATG- 3'	5'- GGTGGAG C AGCACTTC TGATCACC-3'	MspI	5' ---C CGG--- 3' 3' ---GGC C--- 5'	Shahroudi et al., (2011)
MSTN	5´-TGGCGTTA CTCAAAA GCAAA -3´	5´-AACAGCA GTCAGCAG AGTCG-3´	DraI	5' ---TTT AAA--- 3' 3' ---AAA TTT--- 5'	Shahram and Majid (2014)
MHCDRB1	5´-TCTCTGCA GCACATTT CCTGG-3	5´-CTCGCCG CTGCACAG TGAAAC-3´	RsaI	5' ---GT AC--- 3' 3' ---CA TG--- 5'	Ammer et al., (1992)

PCR Amplification

Thermo cycler (Applied Biosystems® Veriti® 96-Well Thermal Cycler Singapore) was conveyed in a final reaction volume of 20µl. A master mix for (46) samples for every gene was readied and an aliquot of 15 µl filled in every PCR tube. Five µl of DNA sample was added to each tube to make the last volume 20 µl [(A GoTaq blue Master Mix 10 µl, 25 units/ml Taq polemerase,each dNTPs is 200mM and MgCl2 is 1.5 mM), 2 µL RFLP primer (forward and reveres), 5µl (50ng) of DNA template and 3µl DNase free water)], (so as to accomplish homogeneity of reagents and decrease the risk of contamination, control reaction were situated up without genomic DNA). DNA was amplified initial denaturation at 95°C for 5 min, followed by 30 cycles consisting of denaturation at 95°C for 30 sec, annealing 60°C for 1 min, extension at 72°C for 1 min, with final extension 72°C for 7 min. The amplification products were size-fractionated in a 2% agarose gel containing Ethidium Bromide in Tris-borate EDTA buffer and visualized under UV transillumination.

PCR –RFLP Analaysis

Following PCR amplification the digested PCR products with the appropriate restriction enzyme for each PCR products separately was carried out to identify if a mutation is present in a DNA samples or not. All digestion component

requirements are shown in Table (2) Mixed delicately by pipetting, close the tube centrifuged for a few seconds in a microcentrifuge (Micro-12, Korea-Hanil Science Industrial). At that point the reactions were incubated for 2-4 hours at the pointed out temperature for the enzyme (generally 37 °C). after high temperature inactivation the product of the reaction digest, 10 µl of the digested samples were taken, included loading buffer, and were separated by electrophoreses on 2.5% agarose gel. One well (poket) every row was utilized for the length standard (100 bp DNA ladder) Bench top, volume 5 µl of length standard was utilized to gauge the size and concentration of the PCR product, the gel was visualized with Ethidum Bromide under the UV Transilluminator (Biostep-UST-20M-8K).

Statistic Analysis

The PROC GLM (General Linear Model) procedure SAS (2002) was used to analyze the data for the genotypes effects for weights at birth, one, two and three months (Weaning Weight) old and average daily gain from birth to weaning analyses were fitted to following equations:

$$Y_{ijklo} = \mu + A_i + S_j + C_k + P_l + \varepsilon_{ijklo}$$

Where: Y ijklo = Body weight at different age and daily of 0^{th} lamb, of i^{th} MSTN (Ai, i=1, BB), of j^{th} CAST (Sj, j= 1, AB and j=2, BB), of k^{th} MHC-DBR1 (Ck, k =1, AA, k=2, AB, k=3, AC and k=4, CC), of l^{th} all genes combinations (Pl, l=1 ,2,3,4,5 and 6), , µ = Population mean, ε_{ijklo} = random error. It was assumed to be normally and independently distributed with mean zero and variance $\delta^2 e$.

RESULTS AND DISCUSSION

Body weight of lambs at different ages

The overall mean of male and female lamb's body weights at birth, one, two, weaning weight and average daily gain from birth to weaning were gives in Table (3).

Table (2): PCR product digestion components with their volume for all genes under study.

Digestion Component	Volume
Sterile, deionized water	5.3 µl
Reaction 10X buffer	2 µl
Acetylated BSA 10µg/µl	0.2 µl
DNA(PCR product) mix by pipetting	7 µl
Appropriate Reaction Enzyme	0.5 µl (5 U)
Final Volume	15 µl

Molecular Profile

Limited molecular genetic information using RFLP markers is available on Iraqi Awassi sheep. Most of the results obtained in this study were the first attempt as a molecular characterization of this breed in Iraq. Fig. (1) shows extracted genomic DNA of total (46) blood samples from Awassi lamb which indicate that the quality of DNA samples are very good and can be used for future work and genetic analysis.

PCR- RFLP TECHNIQUE

PCR-RFLP FOR CAST GENES

The PCR products for the CAST genes were digested with *MspI* restriction enzyme Nanekarani *et al.*, (2011) followed by all samples run on 2.5% agarose gel electrophoresis to detect the genotype for each animal. The digested (622 *bp*) fragment in calpastatin gene gave two genotypes. The BB genotype exhibited 2 fragments of 336 and 286 *bp* and AB genotype exhibited 622,336 and 228 *bp* (Fig. 2). These results indicate that suitable diversity in Awassi sheep breed for calpastatin gene, that can used in future breeding programs. In the present study the diversity is perceived and consisting with

reports on the other breeds (Gabor *et al.*, 2009 and Shahram and Majid, 2014). Allelic and genotype frequency of CAST gene as given in Table (4).

Table (3): Mean ± SD of lamb's weight at different ages.

Lamb	No.	Birth weight (Kg)	Weaning weight (Kg)	Average daily gain (Kg)
Male	25	4.55 ± 0.68	21.38 ± 3.80	0.187 ± 0.03
Female	21	4.25 ± 0.69	19.82 ± 2.94	0.172 ± 0.03
Overall	46	4.4 ± 0.685	20.6 ± 3.37	0.179 ± 0.03

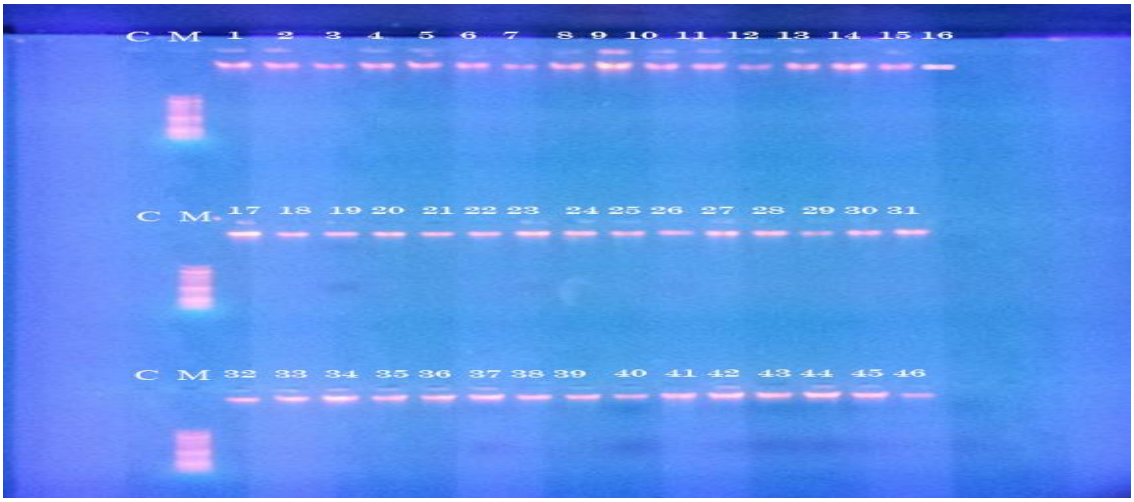

Fig. (1): DNA extraction image for 46 samples Awassi lambs
C: Control without blood sample, M: DNA Ladder 100 *bp*

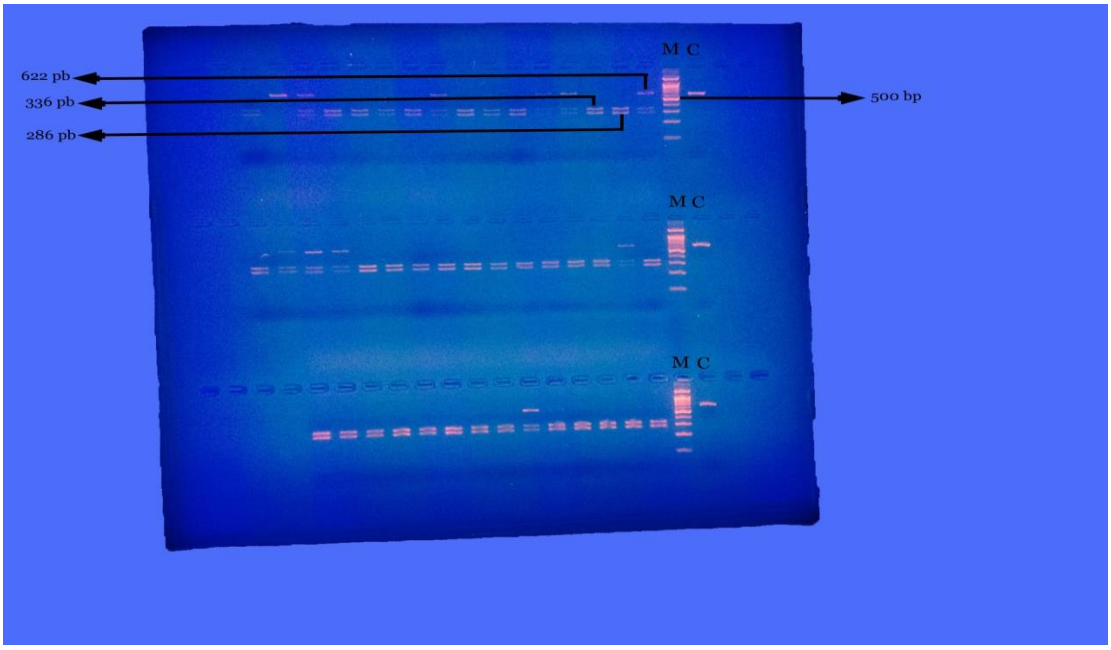

Fig. (2): PCR-RFLP analysis of 622 *bp* fragment of CAST gene by *MspI* enzyme on 2.5% agarose gel electrophoresis to separate bands with stained with 10µl Ethidium Bromide, C=Negative control and M= Molecular size.

Table (4): Allelic and Genotype frequency of CAST gene in Awassi lambs

Genotypes	Allelic frequency		Genotype frequency	
BB(n=35)	B	A	BB	AB
AB(n=11)	0.88	0.12	0.7609	0.2391

PCR-RFLP for MSTN Gene

The *DraI* Restriction enzyme was used to digest the amplified PCR fragments (497 *bp*) according to Xianglong *et al.*, (2008). After that the digested product for each sample was run on 2.5% agarose gel electrophoresis to detect the genotype for all samples. The results show only one genotype for all samples, the BB genotype (427and 70 *bp*) was present in Fig. (3).

PCR-RFLP for MHC-DRB1 Gene

The PCR products for the MHC-DRB1 gene were digested with *RsaI* restriction enzyme (Ashrafi *et al.*, 2014). Samples were migrated on a 2.5 % agarose gel. Four genotypes were observed. Genotype (AB) with three fragments (279, 195 and 84 *bp*), genotype (CC) with two fragments (200 and 79 *bp*). The genotype (AC) with three fragments (279, 200 and 79*bp*) and (AA) with one fragment (279 *bp*), (Fig. 4). Allelic and Genotype frequency of MHC-DRB1 gene are demonstrated in Table (5).

Table (5): Allelic and Genotype frequency of MHC-DRB1 gene in Awassi lambs

Genotypes	*Allelic frequency*			*Genotype frequency*			
AA(n=1)	*A*	*B*	*C*	*AA*	*AB*	*AC*	*CC*
AC(n=39)							
CC(n=2)	*0.4889*	*0.0778*	*0.4333*	*0.022*	*0.067*	*0.867*	*0.044*
AB(n=3)							

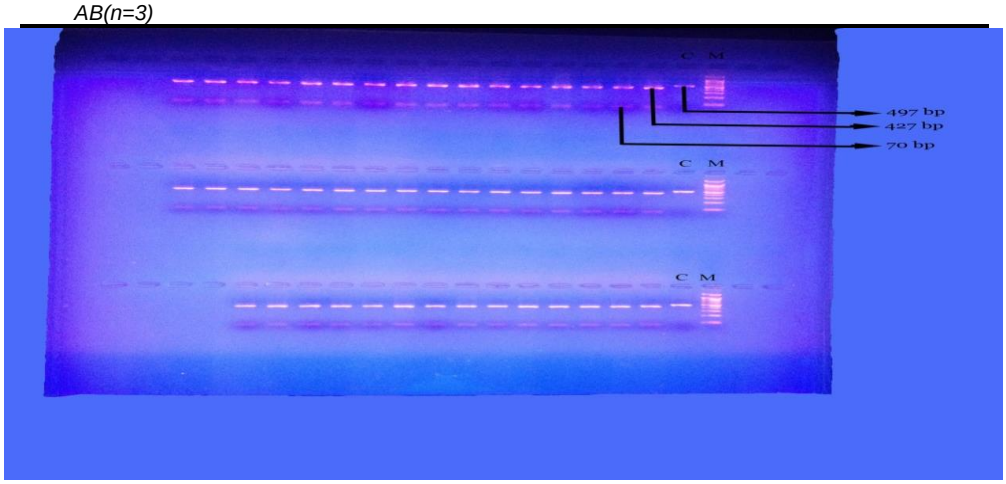

Fig. (3): The results of analysis PCR-RFLP for MSTN gene by restriction enzyme *DraI* on 2.5 % agarose gel electrophoresis. Stained with 10µl Ethidium Bromide.C=Negative control and M= Molecular size.

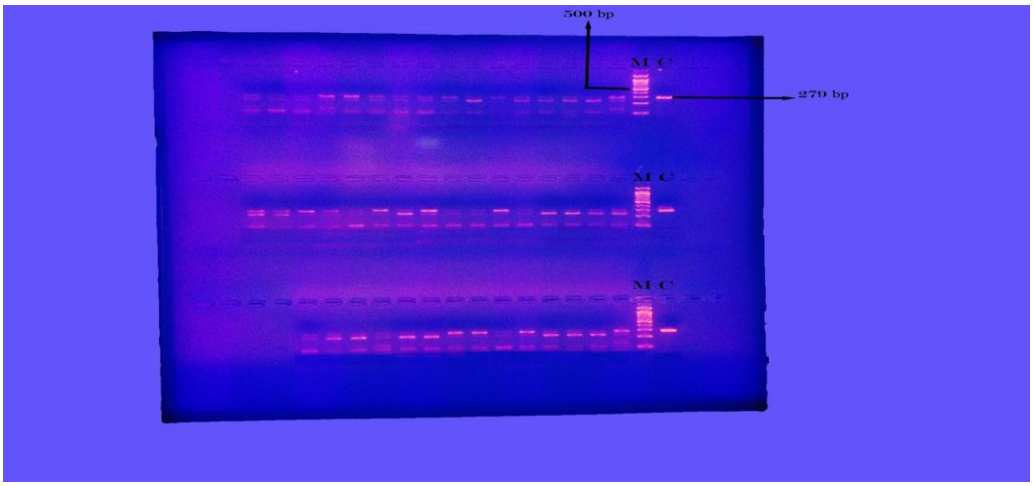

Fig. (4): PCR-RFLP analysis of 279 *bp* fragment of MHC-DRB1 gene by *RsaI* enzyme on 2.5% agarose gel electrophoresis to separate bands with stained with 10µl Ethidium Bromide, C=Negative control and M= Molecular size marker (100 *bp* DNA ladder).

Molecular Markers and Quantitative Traits

The effect of genetic markers on growth traits in Awassi lambs under investigation study are showed in Fig. (5). The CC genotypes of MHC-DRB1 had a positive effect on body weight at all ages except body weight at birth and on average daily gain. The AB genotype at CAST locus gave higher body weight at all ages and higher daily gain compared with BB genotype. Thus the best genotype for lambs at three loci in the present study was BBCCBB which gave 22.15 kg/lambs with 0.197 kg/lamb/day (Table 5). These results reveal that selection can play major role to increase body weight especially weaning weight in Awassi sheep.

This result is similar with Khan *et al.,* (2012), who found in the CAST gene with MN genotype in Balkhi sheep give daily gain higher than the MM genotype at four months' age. Similar results were repoted by Gorlov *et al.,* (2016) for the CAST gene in Salsk sheep and by Ashrafi *et al.,* (2014) in Iranian Makuie sheep.

CONCLUSIONS

CAST and MHC-DRB1 genes were polymorphism while the MSTN gene was monomorphism. Lambs with CC genotype for MHC-DRB1 and lambs with AB genotype for CAST locus gave higher body weight at all ages and higher average daily gain. Lambs with BBCCBB genotypes gave about 1.5 kg/ lamb higher weight at weaning compared with all other genotypes. Thus, the investigation of three specific genes in Awassi lambs indicating the possibility of early selection of animals based on identifying specific genotype to improve the studied trait(s).

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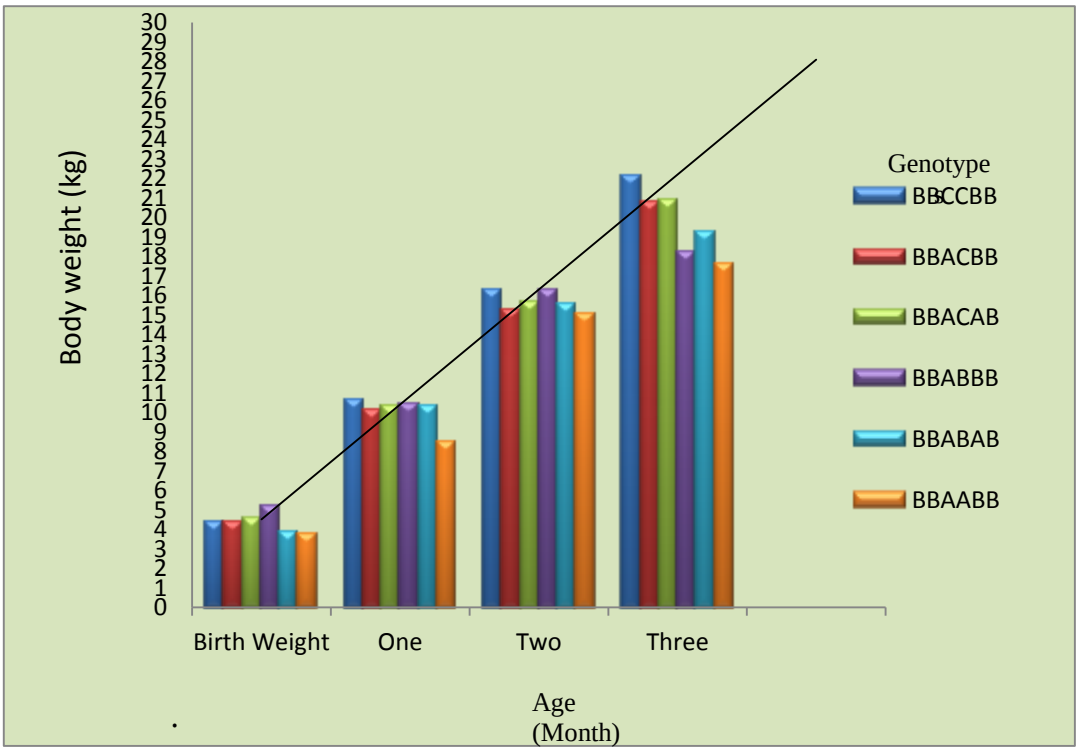

Fig. (5): Effect of loci genotype on the lambs body weight at different ages.

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کارلێکردنی بۆهێڵە تایبەتەکان لەسەر کێشی مەڕی عەواسی عێراقی

پوختە

ئەم توێژینەوەیە ئەنجام دراوە بۆ زانینی کارلێکردنی سێ بۆهێڵی تایبەت (MSTN, CAST, MHC-DRB1)بە کێشی مەڕ لە مەڕی عەواسیدا. پەنجاو دوو بەرخ بەکارهێنرا لە توێژینەوەکەدا. چڕی و پاکی دی ئێن ئەی هەژمارکرا بە بەکارهێنانی ئامێری نانۆدرۆپ کە لە نێوان 10.53 بۆ 53.94 نانۆگرام بۆ هەموو مایکرۆلیترێك وە 1.51 بۆ 2.19 بوو بەدوای یەکدا. ئەنجامی پی سی ئار ئەوەی دەرخست کەوا بۆهێڵی (CAST) وە (MHC-DRB1) جیاوازبوون لە نێوان بەرخەکاندا وە بۆهێڵی (MSTN)یەك شێوازبوو. ئەو بەرخانەی جینۆتایپیان (CC) بوولە بۆهێڵی (MHC-DRB1) وە ئەو بەرخانەی جینۆتایپیان (AB) بوو لە بۆهێڵی (CAST) کێشیان زیاتر بوو لەوانی تر. وە ئەو بەرخانەی کەوا جینۆتایپیان (BBCCBB) یە بۆ هەر سێ بۆهێڵەکان بەرزترین کێشیان داوە لە کاتی لەشیرگرتنەوە کە 22.15 کیلۆگرام بوو بۆ هەر بەرخێك وە بەرزترین زیادکردنی ڕۆژانەیان هەبوو کە 0.197 کیلۆگرام بوو بۆ هەر بەرخێك لە ڕۆژێکدا.ئەم ئەنجامانە ئەوە دەرئەخەن کەوا مەڕی عەواسی تواناییەکی باشی گەشەکردنی هەیەو ئەنجام دانی پرۆسەی هەڵبژاردن بە بەکارهێنانی تەکنیکی بۆماوەزانی گەردی ڕۆڵێکی پۆزەتیفانەو خێرای ئەبێت بۆ بەرەوپێشچوونی ئەم جۆرە مەڕە لە عێراقدا.

تاثير الجينات الخاصة على وزن الجسم في الأغنام العواسية العراقية

الخلاصة

أجريت هذه الدراسة لمعرفة تأثير ثلاث جينات خاصة بالأوزان (MSTN, CAST, MHC-DRB1) في الحملان العواسية. أستخدمت 52 حملا في الدراسة. قيست تركيز و نقاوة الدنا باستخدام جهاز نانودروب وتراوح القيم مابين 10.53 الى 53.94 نانوغرام لكل ملليتر و 1.51 لى 2.19 على التوالي. أوضحت نتائج جهاز البلمرة الحرارية بأن كل من (CAST) و (MHC-DRB1) أعطت حزم مختلفة مابين الحملان ولكن الموقع الجيني (MSTN) أعطت حزمة واحدة لكل الحيوانات تحت الدراسة. الحملان دو تركيب وراثي (CC) للجين (MHC-DRB1) والتركيب الوراثي(AB) للجين (CAST) أعطوا أعلى الأوزان والزيادة الوزنية اليومية عند الفطام. وعلى نطاق المواقع الثلاث فأن التركيب الوراثي (BBCCBB) قد أعطت أعلى معدل لوزن الفطام 22.15 كغم لكل حمل وأعلى معدل للزيادة الوزنية اليومية من الولادة للفطام والبالغ 0.197 كغم لكل حمل يوميا. هذه النتائج تشير الى أن الحملان العواسية لها قدرة وراثية جيدة للنمو وأن الأنتخاب على أساس التقنيات الوراثية الجزيئية لها دور فعال في تسريع عمليات التحسين للأوزان في الأغنام العواسية العراقية.