POLYMORPHISM OF THREE LOCI INFLUENCE ON GROWTH TRAITS IN LOCAL QUAIL

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ABSTRACT

The study aimed to find association between GH, IGF-I and PRL loci with body weight traits in local quail. Three hundred of three lines of local quail were used in the study. The molecular results showed polymorphism for all loci. The five, four and five alleles for GH, IGF-I and PRL locus, respectively were recorded. The allele D of GH, allele A of IGF-I and allele E of PRL locus have positive effect on growth and carcass traits in local quail lines. Based on the results, the CDCDCE genotype of all loci in desert quail significantly gives highest average daily gain (4.75g/ bird/day) and live body weight (208.18 g / bird /day) at marketing age. While the BDBCBE genotype in brown quail gives highest carcass weight (143.55g/bird) and dressing percentage (78.87%). These results show the possibility of rapid improvement and increase of local quail meat production through the selection of distinct alleles and genotypes that affect the growth and production of local quail meat

Keywords: Alleles, genotype, dressing, daily gain, loci

التنوع الوراثي لثلاث مواقع جينية مؤثرة في صفات النمو للسمان المحلي

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المستخلص

هدفت الدراسة إلى إيجاد ارتباط بين مواقع GH و IGF-I و PRL مع صفات وزن الجسم في السمان المحلي. تم استخدام ثلاثمائة من ثلاثة خطوط من السمان المحلي في الدراسة. أظهرت نتائج الوراثة الجزيئية وجود التنوع الوراثي لجميع المواقع.تم تسجيل خمسة وأربعة وخمسة اليلات للموقع الجيني GHو IGF-Iو PRLعلى التوالي. الأليل Dلـ GHوالأليل Aلـ IGF-I والأليل E لـ PRL لهم تأثير إيجابي على صفات النمو والذبيحة في السمان المحلي. بناً ء على النتائج، أعطت التركيب الوراثي CDCDCE لجميع المواقع في السمان الصحراوي أعلى زيادة وزنية يومية (4.75غم / طائر / يوم) ووزن الجسم حي (208.18غم / طائر / يوم) عند التسويق. بينما أعطت التركيب الوراثي BDBCBE للسمان البني اللون أعلى وزن للذبيحة (143.55غم / طائر) ونسبة التصافي (78.87%). تظهر هذه النتائج إمكانية التحسين السريع وزيادة إنتاج لحوم السمان المحلية من خلال اختيار الأليلات المتميزة والأنماط الجينية التي تؤثر على نمو وإنتاج اللحم في السمان المحلي.

الكلمات المفتاحية: الاليل، التركيب الوراثي، نسبة التصافي، الزيادة الوزنية، المواقع الجينية

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INTRODUCTION

Quail is a bird with a tiny body size, rapid growth rate, early sexual maturity, minimal maintenance costs, and a high rate of reproduction (13). Due to its strong similarity to chickens, quails have been utilized in a variety of investigations (1,10). Industrial poultry breeders' and farmers' primary objective is to create high-performing poultry lines. (15). Always two types of data were used to traditionally selection of birds for breeding, they are pedigree and phenotypes. The growth of molecular biology, especially the study of DNA bases and sequences facilitated population studies with genetics and poultry breeding (2). The use of contemporary molecular genetics techniques is an important method for identifying the genotypes of birds and researching prospective genes that may influence poultry production. Also it offers many benefits including selecting birds at an early age and for a wide range of productive traits, rapid detection and accuracy improving productivity (17, 3). Growth characteristics are influenced by a number of genes, but growth hormone (GH) and insulin-like growth factor-I (IGF-I) are the major hormones required to support normal growth (14). Poultry growth hormone and insulin like growth Factor-I are encoded by a gene on chromosome 1 in chicken (2,12). Commonly, high growth rate is determined by growth hormone that produces by GH gene activity and its polymorphism or mutation will affect quail's growth and maturity (22). The IGF-I is a hormone that is structurally related to insulin and important hormone for normal growth in birds and different GH and IGF-I genotypes are predicted to induce different effects on body weight **(**25**)**. Gene polymorphism can be detected in population with PCR-RFLP method (18). A research that detected polymorphisms in the GH gene in Japanese Quail and showed significant association between genotypes with body weight traits, and birds with (AB) significantly higher body and carcass weight compared to AA and BB genotypes (9). The prolactin encoding (PRL) locus is one of the other most important potential loci, an anterior pituitary polypeptide hormone called prolactin was involved in numerous metabolic processes. In addition to controlling growth, differentiation, and breastfeeding, the single-chain polypeptide hormone prolactin also has a significant impact on the cycle of hair development (23). The PRL gene has been linked to a variety of biological functions, making it one of the main physiological prospects for molecular genetic improvement in birds (17,26). The PRL gene located on chromosome 2 in quails (4,11). A research studied the effect of polymorphism of PRL gene on body weight of chickens that had three genotypes AA, AB and BB and showed genotypes significantly associated with body weight at hatch while non-significant at 12 week of age (19). Consequently, purpose of the current research is to investigate the genetic polymorphism of *GH*, *IGF-I* and *PRL* loci and best genotypes of all loci for quail's growth performance by using molecular technique.

METHOD AND MATERIALS

Measuring growth and carcass characteristics

This research was done at the College of Agricultural Engineering Sciences, University of Raparin. A total of 300 quail chicks (100 white, 100 desert, 100) with 10 replications for each color line. The birds were housed in 30 cages, the dimensions for the cages were 45cm × 30cm × 30 cm (length, width and height, respectively). In each cage rearing 10 quail chicks. The birds had free access food and water. The experimental diet contained 23% protein, 2980 Kcal - ME/Kg. The temperature in the environment was 35-37°C for the first week, then dropped by around 2° C weekly until it reached 20-22°C at about 4 weeks of age, when the chicks were completely feathered. light was provided for 24 hours at the first week and decreased two hours weekly until five weeks of age only sixteen-hour lighting and eight-hour darkness. The data was recorded for the studied growth traits during the six weeks. At the end of study at 42 days old, for the study carcass traits from each quail lines four birds were selected from the high body weight (HB) and the low bodyweight (LB), Blood was obtained from each individual quail after each bird was slaughtered by severing the jugular vein.

Sample collection and Genomic DNA extraction: From each bird had one mL of blood samples were drawn, it was afterwards inserted into a 3 mL tube containing the Tris-ethylene di amine tetra acetic acid (EDTA) anti-coagulant. Genomic DNA was extracted from 24 blood sample of local quails by using the (Promega) Genomic DNA Extraction Kit (from blood) according to the manufacturer's instruction as the procedure mentioned in (ReliaPrep™ Blood gDNA Miniprep System). A spectrophotometer and agarose gel electrophoresis were used to measure the amount and quality of the isolated DNA.

The loci selection and primers designing

The loci were chosen in accordance with the previously created chromosomal map by Sasazaki et al (21) Table (1) lists the loci and primers created for this investigation. The GH primers were designed according to Ahmed and Al-Barzinji (1), for the IGF-I were designed according to Hosnedlova *et al,* (8) and for PRL gene were designed according to Cui et *al,* (6).

Table1. Name of loci , Sequences of Primers and Restriction enzyme used in this study

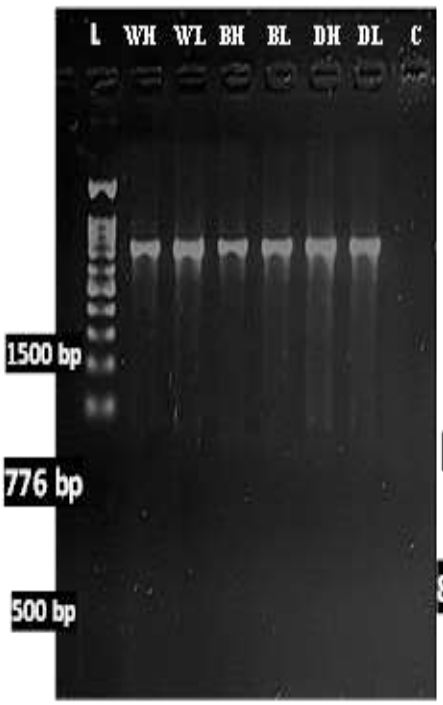
Locus	PCR product size (bp)	Accession Number	Primer Sequence (5'-3')	Restriction Enzyme
GH	776	MN542413	F-ATCCCCAGGCAAACATCCTCG R-CCTCGACATCCAGCTCACAT	*RasI*
IGF-I	813	M74176	F -CATTGCGCAGGCTCTATCTG R -TCAAGAGAAGCCCTTCAAGC	*SmaI*
PRL	154	AB030909.1	F-TTTAATATTGGTGGGTGAAGAGACA R - ATGCCACTGATCCTCGAAAACTC	RasI

PCR amplification

The PCR's overall reaction volume was 40 µL including 20 µL of Taq Master Mix "20mM Tris-HCl (pH8.8), 100mM KCl, 0.2% Triton® X-100, 4mM $MgCl_2$. Protein stabilizer, sediment, loading dye and 0.5mM each of dATP, dCTP, dGTP, and dTTP" , 2µL of each gene's forward and reverse primer, 14 µL of template DNA, and the final volume was finished with 4 µL of water free DNase. The thermal profile included initial denaturation step at 95ºC for 5 min, followed by 30 cycles for IGF-I and PRL and 35 cycles for GH at 95 °C for1 min, annealing at (63.5 °C IGF-I, 57°C PRL and 60°C for GH) for 1 min, elongation at 72 °C for 1 min, and a final extension step at 72 °C for 5 min. Then 2.5% of safe stained agarose gel was employed for the electrophoresis examination of the PCR products. A steady 100 V voltage was applied to the agarose gel for 45 minutes. The bands were seen by using a UV transilluminator. and the gel image was captured by (MultiDoc-It Imaging System with UVP) Figure 1,2 and 3.

DNA Sequencing

A total of 36 PCR amplicons were sent two the commercial company for purification and sequencing (Macrogen Inc. South Korea). (Figure 4).



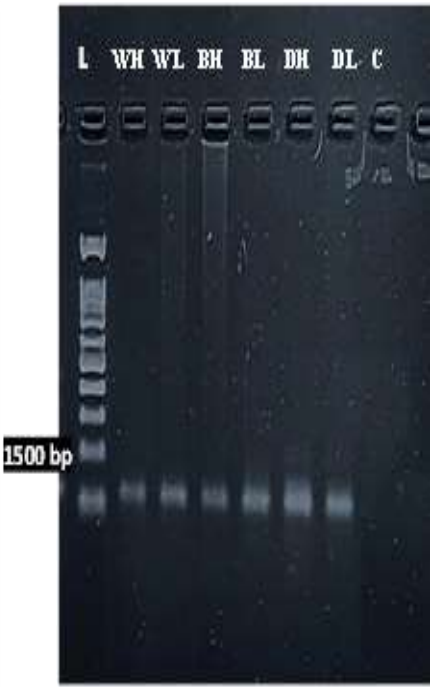
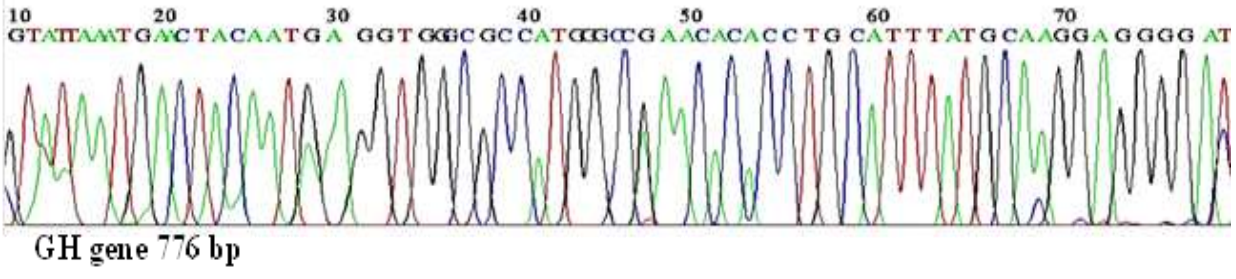


Figure 1. PCR product of GH locus where:- L: DNA marker , Where: WH= White/ Higher production, WL= White / Low production, BH= Brown / Higher production, BL =Brown / Low production, DH= Desert / Higher production DL= Desert / Low production



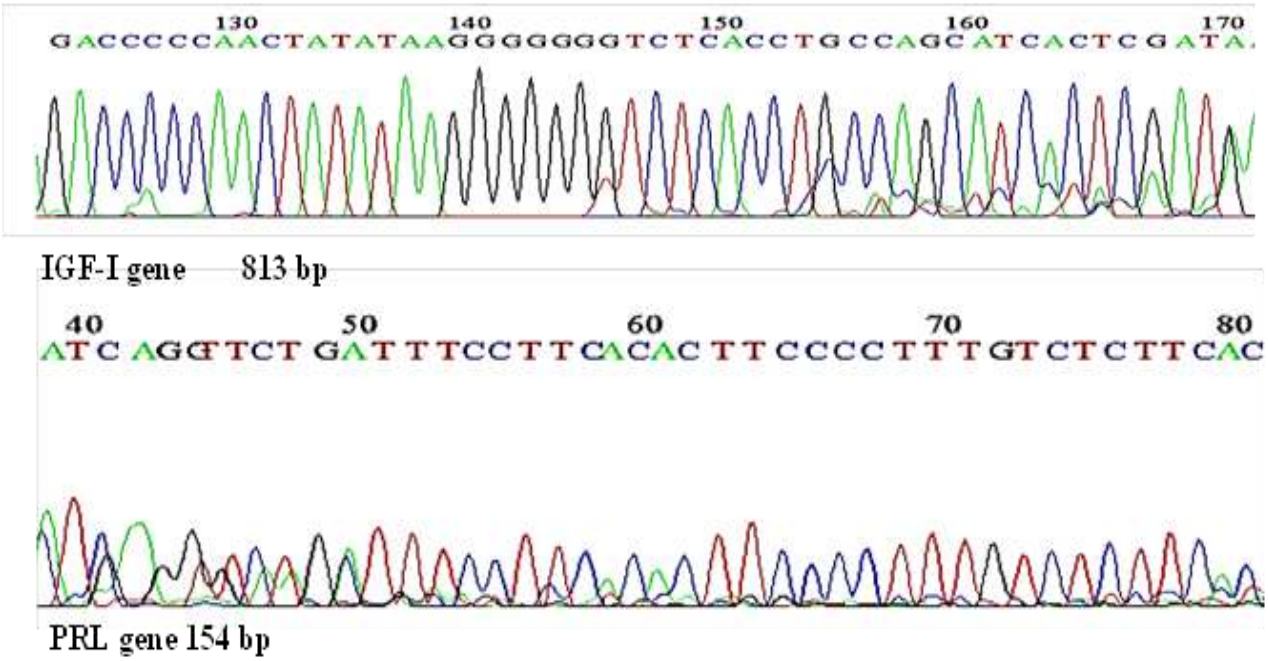


Figure 4. Nucleotide sequence of GH, IGF-I and PRL genes from three different local quail sample the sequence is indicated as the rightward 5' to 3' strand

The restriction fragment length polymorphism (RFLP) technic was used for each individual sequence which cut with specific restriction enzyme that shows in table (1) and the restriction products of specific PCR result samples for each gene of local quail shows in Figures 5, 6, and 7.

Statistical analysis

Direct counting of the bands allowed for the determination of polymorphic loci's genotypes. To analyze the association of genotype with growth and carcass traits the PROC General Linear Model (GLM) procedure SAS, (20) was utilized. Fixed effects research employed the following model:

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

Where: Y ijklo = body weight ,average daily gain ,FCR, carcass weight , dressing percentage of i th GH (Ai, i=1, AA, i=2, BD , i=3, CC,i=4,CD, i=5,CE and i=6,DD), of j th IGF-I(Sj, j= 1, AD, j=2, BC , j=3, BD and j=4, CD), of k th PRL(Ck, k =1, AA, k=2, BE,k=3, CE and k=4, DD) of l th all genes combinations (Pl, l=1 ,2,3,4,5 and 6), μ = Population mean, εijklo = random error. It was assumed to be normally and independently distributed with mean zero and varianceδ 2e. In order to calculate the significant difference among means, Duncan's multiple ranges for a means were employed.

RESULTS AND DISCUSSION

Polymorphism Detection

The GH, IGF-I and PRL fragment sequences were digested by *RsaI, SmaI* and RsaI, respectively (Figure 5, 6 and 7). The RFLP pattern of GH locus observed five different alleles (E, B, D, C and A) and six genotypes (DD, BD, CC, CD, CE and AA), while for the IGF-I locus four different alleles (A, B, C and D) and four genotypes (AD, BC, BD and CD) were found and PRL locus produces five kinds of alleles (A, B, C, D and E) and four genotypes (AA, BE, CE and DD),(Table,2). The results agree with İlhan (9) were determinate three genotypes of Japanese quail for GH/ MspI they were AA (539 bp, 237 bp), BB (776 bp) and AB (776bp, 539bp, 237 bp). This finding had come in agreement with Hosnedlova *et al* (8) who also studied the IGF1/Hinf-I of chicken showed only two genotypes, AA (378 bp, 244 bp , 191 bp), and AC (622 bp , 378 bp , 244 bp and 191 bp). The results agree with Li *et al* (16) the fragments were obtained for PRL/DraI polymorphism in Gaoyou duck: 518 bp and 47bp for AA genotype, 518 bp,309 bp,209 bp and 47 bp for AB genotype, and 309 bp, 209 bp and 47 bp for the BB genotype.

Table 2. Band number and fragments size (Bp) for GH, IGF-I and PRL genes in local quails

Quail Line groups	GH			IGF-I			PRL		
	Genotype	No. of band	band Size bp	Genotype	No. of band	band Size bp	Genotype	No. of band	band Size bp
WH	DD	3	280+ 268+ 228	AD	3	596+164+53	CE	2	54+100
WL	CE	4	338+ 301+ 103+ 34	BD	4	419+38+135+221	AA	1	154
BH	CC	2	377 + 399	BC	3	427+38+348	DD	2	80+74
BL	BD	2	556 + 220	BC	3	417+38+358	BE	2	44+110
DH	CD	3	415+ 269 + 92	CD	3	391+192+230	CE	2	55+99
DL	AA	1	776	BC	3	414+56+343	CE	2	56+98

Where: WH= White/ Higher production, WL= White / Low production, BH= Brown / Higher production, BL =Brown / Low production, DH= Desert / Higher production DL= Desert / Low production.

Marker assisted selection (MAS)

The analysis results of association between the GH, IGF-I and PRL loci polymorphisms and growth performance traits of three local quail lines are shown in Table 3. In accordance to the genotype for all genes in the study showed a significant association with the growth performance traits of local quails, the highest body weight at 42 days (208.18g/bird) and average daily gain (4.75 g/ bird/day) were recorded for CDCDCE genotype in desert quail. while the lowest body weight (171.24 g/bird) and average daily gain (3.88 g/ bird/ day) were recorded in CEBDAA genotype of

white quail line. Non-significant among genotypes were found of feed conversion ratio (FCR), and the best FCR were recorded in brown quail with CCBCDD genotype (3.3 g feed / 1 g meat). Significant association results were observed by Ahmed and Al-Barzinji (1) between the polymorphisms of SEMA3E, TLX and GH loci with growth traits in local quail which best body weight genotype was AAABAA in desert quails. Regards to IGF-I gene the results disagree with results of El-Bayomi *et al***.** (7) that observed no significant link between IGF-I genotypes and body weights of Japanese quail. Also Shulika and Kulibaba (24) found a non-significant correlation between chicken PRL gene polymorphism and growth traits.

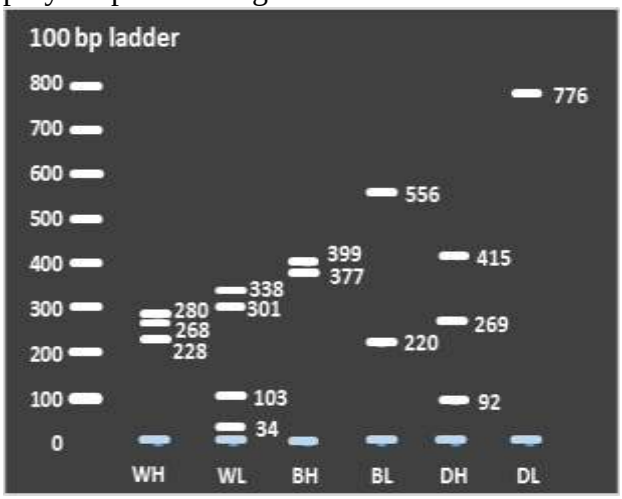


Figure 5. Digestion of PCR product of GH with *RsaI*

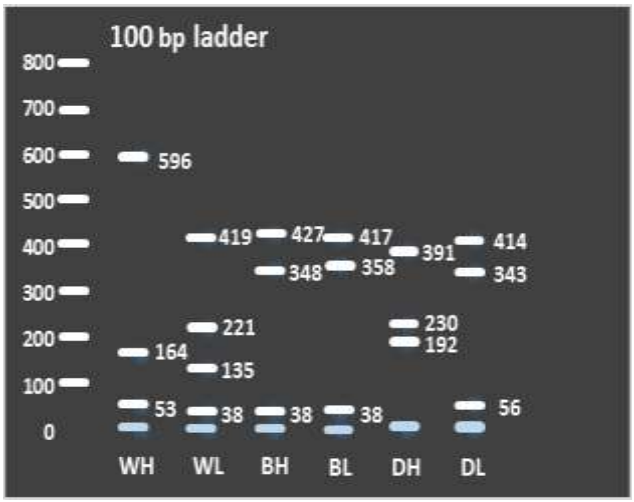


Figure 6. Digestion of PCR product of IGF-I with *SmaI*

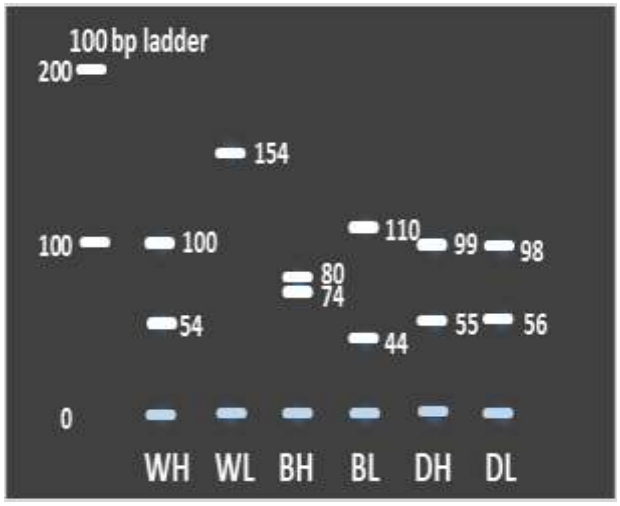


Figure7. Digestion of PCR product of PRL with *RsaI*

The effects of genotypes of the GH, IGF-I and PRL genes on carcass traits in local quail are shown in Table 4. The best genotype of carcasses traits is BDBCBE recorded in brown quail for carcass weight (143.55 g/bird) and dressing percentage (78.87%). These results which is consistent with what found by İlhan. (9) for GH locus who observed a significant association between genotypes and carcass weight, the quail with AB genotypes had higher carcass weight compared to AA and BB genotypes. A similar founding reported by Attarchi *et al* (5) were indicated a significant association between genotypes of IGF-I and GH genes with carcass weight of chicken, and birds with (--) genotypes for IGF-I gene had significantly higher carcass weight compared to the other genotypes (+- and ++), while for GH gene (++) genotype significantly superior for carcass weight compared with the other genotypes. Our results disagree with Shulika and Kulibaba (24) that showed non-significant association PRL gene genotypes with carcass weight in chicken.

Table 3. Relationship between three loci genotype with growth traits in local quail

Colors	Line	GH × IGF-I × PRL	BW(g)	DWG (g)	FCR
White	High production	DDADCE	200.34±3.73 abc	4.57±0.08 ab	3.6±0.33 a
	Low production	CEBDAA	171.24±10.98 c	3.88±0.26 c	3.8±0.22 a
Brown	High production	CCBCDD	207.2±11.03 a	4.74±0.26 a	3.3±0.22 a
	Low production	BDBCBE	182.27±7.24 bc	4.15±0.17 bc	3.5±0.22 a
Desert	High production	CDCDCE	208.18±4.99 a*	4.75±0.11 a	3.4±0.33 a
	Low production	AABCCE	188.81±4.66 abc	4.3±0.11 abc	3.35±0.24 a

***Different litters within each column differ significantly at level (*P≤0.05*) ; ,BW= Body weight (g) DWG= Daily weight gain(g), FCR=Feed contrition ratio (g).**

Table 4. Relationship between three loci genotype with carcass traits in local quail

Colors	Line	GH × IGF-I × PRL	CW	DP
White	High production	DDADCE	141.07±2.93 a	74.57±0.91 b
	Low production	CEBDAA	128.25±3.03 b	74.27±1.5 b
Brown	High production	CCBCDD	131.95±4.87 ab	76.25±0.84 b
	Low production	BDBCBE	143.55±5 a*	78.87±0.78 a
Desert	High production	CDCDCE	138.12±4.8 ab	75.57±0.06 b
	Low production	AABCCE	135.47±2.8 ab	73.85±0.67 b

***Different litters within each column differ significantly at level (P≤0.05) CW= Carcass weight(g), DP= Dressing Percentage (%)**

CONCLOSION

We conclude from the results of the current study that the best combination and genotypes of weight characteristics in local quail is the CDCDCE genotype in desert quail (body weight: 208.18 g/bird and average daily gain: 4.75 g/bird/day). The best genotype for FCR (3.3 g feed/1 g meat) and carcass traits (carcass weight: 143.55 g/bird and dressing: 78.87%) are CCBCDD and BDBCBE, respectively in brown quail. Thus selecting these genotypes at an early age of quail to be parents to the next generation shortens the generation period and accelerates genetic improvement of growth traits of this type of local birds

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