

CROSSBREEDING EFFECTS AND POLYMORPHIC ASSOCIATIONS OF GENOTYPES OF GH GENE WITH GROWTH TRAITS IN RABBITS

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A simple crossbreeding experiment between two new synthetic rabbit lines (APRI and Moshtohor) was conducted to estimate the crossbreeding effects (direct additive, maternal additive and heterosis) on post-weaning body weights (BW) at 4, 6, 8, 10 and 12 weeks of age and daily weight gains (DG) during the intervals from 4 to 6, 6 to 8, 8 to 10 and 10 to 12 weeks, and to detect the polymorphic associations of the genotypes of GH gene with post-weaning body weight and daily gain traits. Four genetic groups of APRI (A-line), Moshtohor (M-line) and their simple F₁ cross (1/2A1/2M) in addition to V-line rabbits (as a reference population) were used in this study.

PCR-RFLP technique was applied using BstUI restriction enzyme SNP for genotyping the growth hormone gene (GH) (TT, TC and CC) genotypes. Heritabilities estimated by VCE program for body weights and daily weight gains were mostly low or moderate and ranging from 0.05 to 0.18. The percentages of direct additive effects on body weights were significantly ($P < 0.01$) in favour of Moshtohor line by 2.7, 4.3, 1.4, 2.6 and 2.9 % at 4, 6, 8, 10 and 12 weeks and by 8.7, 15.9 and 18.6 % in daily weight gains at DG4-6, DG6-8 and DG8-10, respectively. The percentages of maternal effects on body weights at 4, 6, 8, 10 and 12 weeks were also significantly in favour of Moshtohor line by 2.8, 5.4, 3.5, 3.5 and 3.1%, respectively and ranged from 1.8 to 15% for daily weight gains in weight. The percentages of direct heterosis were significantly positive by 3.1, 8.1, 8.2, 5.2 and 3.8 % for body weights at 4, 6, 8, 10 and 12 weeks and by 13.0, 7.8 and 9.6% for daily weight gains at DG6-8, DG8-10 and DG10-12, respectively. The associations of the genotypes of GH gene were polymorphic and had significant effects on most growth traits ($P < 0.05$). There were significant differences ($P < 0.05$) between the

generalized least square means of the different genotypes (*TT*, *CC* and *TC*) of *GH* gene for body weights at 4, 6, 8, 10 and 12 weeks of age in *V*-line, *M*-line, *A*-line and $\frac{1}{2}A\frac{1}{2}M$ cross rabbits. The *TC* genotype was positively associated with an increase in body weights of *V*-line, *M*-line and *A*-line rabbits, while the *TT* genotype indicated an increase in body weights of $\frac{1}{2}A\frac{1}{2}M$ rabbits. The significant associations between *GH* gene and growth traits confirmed that this gene could be a candidate gene and may be used in Marker-assisted selection (MAS) to improve growth performance in rabbits.

Conclusively, *GH* gene could be used as a candidate gene in genetic improvement programs to improve growth performance in rabbits.

Keywords: Rabbits, Growth, Crossbreeding effects, Genotyping, *GH* gene, PCR-RFLP.

Crossbreeding is usually used to improve the overall production efficiency by using some breeds which have high genetic merit for different economic traits. In crossbreeding experiments performed in rabbits, most of the Egyptian and non-Egyptian studies (*e.g.* Piles *et al.*, 2004; Abou Khadiga *et al.*, 2008; Iraqi *et al.*, 2008; Youssef *et al.*, 2009; Khalil and Al-Homidan., 2014) showed significant direct additive and maternal genetic effects along with significant heterotic effects on body weights and gains at different ages. Until now, few studies have surveyed candidate genes for reproduction and growth traits in rabbits (Peiro *et al.*, 2008a; Merchan *et al.*, 2009; Garcia *et al.*, 2010).

The candidate genes have successful approaches in identifying several DNA markers associated with production traits in rabbits. There were significant associations between the genotypes SNP and body weights. In this concept, the growth hormone gene (*GH*) located on chromosome 19, growth hormone receptor gene (*GHR*) located on chromosome 11, progesterone receptor gene (*PGR*) located on chromosome 1, fibroblast growth factor gene (*FGF*) located on chromosome 3, insulin-like growth factor 1 and 2 genes (*IGF1* and *IGF2*) located on chromosome 4, myostatin gene (*MSTN*) located on chromosome 7 and melanocortin 4 receptor gene (*MC4R*) located on chromosome 9 have shown significant associations with body weights in rabbits (Inoue *et al.*, 2006; Fontanesi *et al.*, 2008; Lu *et al.*, 2011; Zhang *et al.*, 2012; Fontanesi *et al.*, 2012; Peng *et al.*, 2013; Sahwan *et al.*, 2014; Wu *et al.*, 2015; Othman *et al.*, 2015; El-Aksher *et al.*, 2016; El-Sabrout and Aggag, 2017; Migdal *et al.*, 2018). The growth hormone gene (*GH*) alters the metabolism of carbohydrates, proteins and lipids and promotes postnatal growth of mammals exerting direct or indirect effects on numerous tissues (Fontanesi *et al.*, 2012).

The technique of polymerase chain reaction (PCR) based on restriction fragment length polymorphism (PCR-RFLP) was recently used by Amalianingsih *et al.* (2014) and Hussein *et al.* (2015) for analysing single nucleotide polymorphisms (SNP) of the *GH* gene in rabbits where a significant association between the SNP and marketing weight was detected. El-Sabrou and Aggag (2017) examined DNA parts of six growth genes: growth hormone (GH), growth hormone receptor (GHR), melanocortin 4 receptor (MC4R), phosphoglycerate mutase (PGAM), myostatin (MSTN), and fibroblast growth factor (FGF) for two rabbit lines (V line and Alexandria) to investigate the associations between SNP of these genes and body weight at marketing. *GH* gene mutations are described in several species, however, the studied concerning its variability and associations with economic traits in rabbits are scarce.

So, that aims of the current study were: (1) to estimate crossbreeding effects (*i.e.* direct additive, maternal additive and heterotic effects) on some growth traits in a crossbreeding experiment between APRI line (A) and Moshtohor line (M), and (2) to detect the associations between (C>T) SNP genotypes of *GH* gene and growth traits in rabbits.

MATERIALS AND METHODS

A crossbreeding experiment performed and animals used:

A crossbreeding experiment between APRI line bucks (A) with Moshtohor line does (M) was carried out in the rabbitry of the Faculty of Agriculture, Benha University, Egypt to get $\frac{1}{2}A\frac{1}{2}M$ cross. This experiment was conducted during the period from September 2015 until December 2017 to determine the crossbreeding effects on growth traits.

The animals used in this experiment were Moshtohor line, APRI line, $\frac{1}{2}A\frac{1}{2}M$ and V line as a reference population. APRI line was developed in the Animal Production Research Institute, Ministry of Agricultures, Egypt (Youssef *et al.*, 2009). The synthesis was conducted by crossing Red Baladi bucks (B) with V line does (V) followed by selection for litter weight at weaning in three subsequent generations and after F3, the animals were called APRI line consisting of $\frac{1}{2}V\frac{1}{2}B$. Moshtohor line was established in the Department of Animal Production, Faculty of Agricultures, Moshtohor, Benha University, Egypt (Iraqi *et al.*, 2008). The synthesizing pattern of Moshtohor line was similar to that for APRI line, but with using Sinai Gabali bucks (S) instead of Red Baladi bucks. Consequently, this line is formed from 50% Sinai Gabali and 50% V line ($\frac{1}{2}S\frac{1}{2}V$) where selection was practiced for litter weight at weaning and individual weight at 56 d and the animals were kept in the

rabbitry of Benha University. The V line is a maternal line selected for litter size at weaning by Animal Science Department, Universidad Politécnic de Valencia, (UPV), Valencia, Spain. The interest of using line V lies in several features; first it has long history of selection in Valencia where the climate is not widely different from the weather of Delta of Nile in Egypt (Garcia and Baselga, 2002), second it has good performance in Saudi Arabia and had confirmed the fact that it has adaptable pattern in heat stress conditions (Khalil *et al.*, 2005).

Mating practiced:

Mating was occurred at random within each genetic group, avoiding full and half sibs matings as well as parent offspring matings. The total number of weaned bunnies, sires and dams were 1201, 179 and 261, respectively (Table 1). Age of the does and bucks ranged between 4.5 and 5 months at first time of mating. Culled bucks and does or dead ones during the experimental period were replaced randomly by their replacements from the same line and from the original stock. The ratio of the experimental buck to does was 1:3 by using natural mating. Sire-daughter, full and half-sibs matings were avoided. Each doe was transferred to the assigned buck to be mated and returned back again to her own cage. On the day 10 post mating, each doe was palpated to detect pregnancy. Doe that was not pregnant would returned to the same mating buck to be re-mated and returned every other day thereafter until a service was observed. Likewise, does were re-mated after kindling by the same assigned buck.

Table 1: Number of animals, sires and dams categorized according to their genetic groups

Animal genetic group	Buck genetic group	Doe genetic group	Number of bunny weaned	Number of sires used	Number of dams used
V	V	V	439	60	90
A	A	A	272	47	58
M	M	M	312	48	73
½A½M	A	M	178	24	40
Total			1201	179	261

V=V line, A=APRI line, M=Moshtohor line.

Housing and feeding:

The rabbits were raised in one floor rabbitry, oriented east to west where windows and fans were oriented to control the ventilation. The rabbitry was

equipped with regular electric heaters in winter to keep the minimum degree of temperature at 15°C. In summer, there are fans and forced air-cooling to keep the maximum temperature at 35°C. Breeding bucks and does were housed individually in wired cages with standard dimensions (60 x 40 x 35 cm of length x width x height) and arranged in flat deck batteries. In the rabbitry, temperature ranged from 12 to 19°C in winter with the relative humidity ranged from 40 to 80 % and photoperiod was at 16 L: 8 D. Each maternity cage was supplied with a galvanized steel nest boxes. Cages and nest boxes were cleaned and disinfected regularly after each kindling and urine and feces were cleaned every day morning. On the 25th day of pregnancy, the nest boxes were supplied with thick layer of rice straw, which was placed in the bottom of the nest box to help the doe in preparing a warm comfortable nest for her bunnies. Litters were examined and counted within 12 hours after kindling and were checked and examined every morning during the suckling period to remove the dead bunnies. The bunnies were weaned, sexed, ear tagged, and transferred to standard progeny wire cages at 28 days post kindling.

The breeding animals were fed *ad libitum* all over the experimental period on a pelleted commercial ration; pellets were cylindrical in form (1-2 cm in length and 0.4 cm in diameter). The ration was composed of 23 % barley , 19 % wheat bran, 24 % soybean meal , 21 % berseem hay , 13 % yellow corn , 1 % limestone, 0.5 % table salt, 14 kg di-calcium phosphate/ton, 1 kg minerals mixture/ton, 1 kg anti-coccidian/ton, 1 kg anti-toxicity/ton, provided 18.01% crude protein, 13.7% crude fiber and 2.5% fat (digestible energy = 2500 to 2700 kc/kg feed). Berseem "*trifolium alexandrinum*" was provided in winter months to all breeding animals to increase milk production of rabbit does and improve livability. All the animals were kept in the same house and reared under the same environmental conditions in every stage of productivity. Fresh drinking water was available all the time.

Data of growth traits:

Data were recorded for individual rabbits body weights (BW) in four genetic groups (V line, APRI line, M line and ½A½M cross) at 4, 6, 8, 10, and 12 weeks of age. Daily weight gains (DG) were calculated during the age intervals from 4-6, 6-8, 8-10 and 10-12 weeks of age.

Statistical models for estimating the crossbreeding genetic effects:

Data of body weights and daily weight gains were analyzed using the following multi-trait animal model:

$$y = Xb + Z_a u_a + e \quad \text{(Model 1)}$$

Where y = vector of the observed growth trait for the weaned rabbit; b= vector of the fixed effects of genetic group of progeny (four levels, see Table 1), sex

(males and females), year-season of birth (eight levels), parity (five levels) and litter size in which the animal was born (three levels); X and Z_a are incidence matrices corresponding to fixed and random additive effects of the rabbit (u_a), respectively; e = vector of random residual effects.

The variance components of random effects and heritabilities were estimated by software: VCE6 (Groeneveld *et al.*, 2010). The variance components were used to solve the corresponding mixed model equations, obtaining solutions for the genetic group effects and their error variance–covariance matrix using the PEST software (Groeneveld, 2006). According to the theory of Dickerson (1992), the solutions of the crossbreeding genetic group effects were obtained using the procedure of Generalized Least Squares (GLS) and applying the following linear model:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{e}, \mathbf{Var}(\mathbf{y}) = \mathbf{V} \quad \text{(Model 2)}$$

Where: y = Vector of the estimated genetic groups solutions; X = Incidence matrix; b = Vector of estimable crossbreeding genetic effects; e = Vector of random error; V = Error variance–covariance matrix of y .

The coefficients relating the genetic crossbreeding effects to the means of the genetic groups (Table 2) were estimated according to the model of Dickerson (1992) and using CBE software (Wolf, 1996). In the experiment of crossing APRI bucks with Moshtohor does, the crossbreeding parameters representing the differences between the genetic groups were estimated in terms of direct additive effects ($G^I = G^I_A - G^I_M$), maternal additive effects (G^M) and direct heterosis (H^I). Thus, we have three parameters to be estimated (the vector called b -vector):

$$\mathbf{b} = [(G^I_A - G^I_M) \ G^M \ H^I]$$

The solutions of \mathbf{b} were calculated by the method of Generalized Least Squares (GLS) using the following equation:

$$\hat{\mathbf{b}} = (\mathbf{X}'\mathbf{V}\mathbf{X})^{-1}\mathbf{X}'\mathbf{V}\mathbf{y}$$

Where: \mathbf{X} was the matrix of coefficients of estimable crossbreeding effects, \mathbf{V} = Generalized error variance–covariance matrix, with the variance–covariance matrix of the estimate of \mathbf{b} being,

$$\mathbf{Var}\hat{\mathbf{b}} = (\mathbf{X}'\mathbf{V}\mathbf{X})^{-1}$$

The matrix used to test the significance of the crossbreeding effects is presented in Table 2.

Molecular genetic analyses:

Blood sampling and DNA extraction:

Two hundred and eleven rabbits belong to V line, APRI line, M line and $\frac{1}{2}A\frac{1}{2}M$ cross were used in molecular genetic analyses. The samples were taken from animals with the least relationship with avoiding full sibs to decrease the

Table 2: Genetic groups of animals with their sires and dams and coefficients of the matrix relating the means of the genetic groups with crossbreeding effects

Genetic group			Mean	Coefficients of the matrix				
Animal	Sire	Dam		G_A^P	G_M^P	G_A^M	G_M^M	H^I
A	A	A	1	1	0	1	0	0
M	M	M	1	0	1	0	1	0
$\frac{1}{2}A\frac{1}{2}M$	A	M	1	0.5	0.5	0.5	0.5	1

G_A^P and G_M^P = Direct additive genetic effects for APRI and Moshtohor lines, respectively; G_A^M and G_M^M = Maternal genetic effects for the APRI and Moshtohor lines, respectively; H^I = Direct heterosis.

genetic relationships between the genotyped animals. Approximately 3-5 ml venous blood sample per animal was obtained from the rabbit ear vein by 2-gauge 1.5-injection needle into tubes containing EDTA as anticoagulant. The blood samples were preserved in ice tank till reaching the laboratory and were then kept in freezer at -20°C . Genomic DNA was extracted from leukocytes using the Gene Jet Whole Blood Genomic DNA purity Mini Kit (Cat No. #K0781, Thermo Scientific).

Amplification by polymerase chain reaction (PCR):

PCR was done for amplification of part of the 5' untranslated region and part of exon 1 of the growth hormone gene (*GH*) with expected amplicon size of 231 bp. According to Fontanesi *et al.* (2012), the primers used in the amplification were: the forward primer 5'- GTATAGTGGGATGGGGTTGG - 3' and the reverse primer 5'- TTACGCTCCCATTCAGAAGC -3 (Table 3). PCR amplifications were carried out in 50 μl reaction mixture composed of 4 μl genomic DNA (100 ng/ μl) as a template, 10 pmol of each primer, 2mM dNTP' (dATP, dCTP, dTTP and dGTP; ABgene, Surrey, UK), 10X PCR buffer, 25 mM MgCl_2 , and 1 unit *Taq* DNA polymerase. A master mix was prepared in a 2 ml tube according to the number of PCR reactions to be performed, with an extra reaction included to compensate the loss part of the solution due to frequent pipetting. An aliquot of 46 μl master mix solution was dispensed in each PCR tube (0.2 ml tube), containing 4 μl of the appropriate template DNA. Accordingly, the PCR reaction components are presented.

Thermal cycling was carried out by initial denaturation at 95°C for 5 min, followed by 35 cycles each at 95°C for 30 seconds, annealing temperature at 58°C for 30 seconds, extension temperature at 72°C for 30 seconds and final extension at 72°C for 10 min., then the samples were held at 4°C .

Table 3: Primer sequence and PCR-RFLP assay conditions for genotyping SNP of *GH* gene

Gene(Gene Bank Accession no.)	Location	Primer sequences (forward/reverse)	PCR Product size (bp)	Annealing temp per time (°c/s) ^a	Restriction Enzyme
<i>GH</i>	Chromosome 19, NC_013687.1	5'- GTATAGTGGGATGGGGTTGG-3' 5'- TTACGCTCCCATTCAGAAGC -3'.	231	58/30	<i>BstUI</i>

^a PCR annealing temperature and time for primer.

The amplified DNA fragments were separated on 2 % agarose gel, stained with ethidium bromide, visualized on a UV Transilluminator and photographed by gel documentation system (Alpha Imager M1220, Documentation and Analysis, System, Canada).

Genotyping of GH gene using PCR-RFLP technique:

The c.-78C>T SNP of the growth hormone gene (Fontanesi *et al.* 2012) was genotyped by PCR-RFLP using *Bsh1236I* (*BstUI*) restriction enzyme (Fermentas, Vilnius, Lithuania). The RFLP technique was carried out in reaction volume 20 µl consisted of: 7 µl H₂O, 2 µl buffer, 10 µl PCR product and 1 µl restriction enzyme. Digested fragments were visualized by electrophoresis on 3 % agarose gel stained with ethidium bromide at 100 V in 1x TAE and visualized on a UV Transilluminator and photographed by gel documentation system. The 50 bp DNA step ladder (Promega) was included in each run.

Model for detecting the associations between genotypes of GH gene and growth traits:

For the associations between the SNP genotypes of *GH* gene and post-weaning growth traits in each genetic group separately, the effects of genotype C/T SNP on different growth traits were estimated using PEST software (Groeneveld, 2006) and applying the same multi-traits animal model defined in Model 1 (in matrix notation) after adding the fixed effect of the *i*th C/T SNP genotype of *GH* gene (three genotypes; TT, CC and TC). The method of Generalized Least Squares (GLS) was used to get the polymorphic associations between genotypes of *GH* gene and growth traits.

RESULTS AND DISCUSSION

Actual means and variations:

Actual means, standard deviations (SD) and coefficients of variation (CV%) for body weights at 4, 6, 8, 10 and 12 weeks of age and daily weight

gains (DG) during the intervals from 4 to 6, 6 to 8, 8 to 10 and 10 to 12 weeks of age across all genetic groups were presented in Table 4. The overall means of body weights were 490, 780, 1085, 1330 and 1670 g at 4, 6, 8, 10 and 12 weeks of age and daily weight gains were 20.5, 21.8, 18.2 and 24.2 g at 4-6, 6-8, 8-10 and 10-12 weeks, respectively. Youssef *et al.* (2009) estimated that the average body weights at 4, 6, 8, 10 and 12 weeks of APRI line to be 501,740, 1028, 1323 and 1651g and the daily weight gains were 17.0, 20.5, 20.9 and 23.2 g at 4-6, 6-8, 8-10 and 10-12 week, respectively.

Table 4: Actual means, standard deviations (SD), coefficients of variation (CV), and ranges for growth traits across all genetic groups

Trait	Number of records	Mean	SD	CV	Minimum	Maximum
<i>Body weights at:</i>						
4 weeks (g)	1201	490	60.93	12	310	685
6 weeks (g)	1164	780	80.43	10	530	1010
8 weeks (g)	1116	1085	112.16	10	760	1350
10 weeks (g)	1058	1330	96.22	7	1000	1820
12 weeks (g)	984	1670	138.46	8	1230	2000
<i>Daily gains at:</i>						
4-6 weeks (g)	1163	20.51	7.04	34	1.4	50
6-8 weeks (g)	1115	21.84	8.56	39	0.7	45.7
8-10 weeks (g)	1046	18.17	7.91	44	0.7	52.1
10-12weeks (g)	979	24.16	9.20	38	0.7	57.1

The ranges between minimum and maximum values in body weights at 4, 6, 8, 10 and 12 weeks across all genetic groups of rabbits were high, being 310 to 685, 530 to 1010, 760 to 1350, 1000 to 1820 and 1230 to 2000 g, respectively and from 0.7 to 57.1g for daily weight gains. The coefficients of variation for body weights were moderate, ranging from 7 to 12%, while the coefficients for daily weight gains were high ranging from 34 to 44%.

Body weights and daily gains in different genetic groups:

Least square means of body weights in $\frac{1}{2}A\frac{1}{2}M$ cross were higher than APRI line and lower than Moshtohor line at 4, 6, 8, 10 and 12 weeks of age (Table 5). These averages were lower than those values obtained for line V reared in Spain (*e.g.* García *et al.* 2000). These averages of $\frac{1}{2}A\frac{1}{2}M$ cross rabbits were higher than those of APRI line intensified in Egypt (Youssef *et al.*, 2009). Moreover, body weight at 4, 8 and 12 week of $\frac{1}{2}A\frac{1}{2}M$ cross rabbits were in agreement with Iraqi *et al.* (2008) who found that the average body weights at 4, 8 and 12 weeks was 589, 1193 and 1676g in Moshtohor line rabbits.

Table 5: Generalized least square means \pm standard errors for body weights (BW) and daily gains (DG) as affected by genetic groups

Traits	Genetic groups							
	V Line		M Line		A Line		$\frac{1}{2}A\frac{1}{2}M$	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
<i>Body weights at:</i>								
BW4	475 ^d	15.8	520 ^a	20.09	490 ^c	18.81	505 ^b	21.38
BW6	780 ^b	29.6	800 ^a	27.28	755 ^d	25.73	775 ^c	29.17
BW8	1125 ^a	40.6	1085 ^b	37.17	1040 ^c	35.03	1050 ^c	40.15
BW10	1325 ^b	89.6	1370 ^a	78.83	1310 ^d	74.03	1320 ^c	85.8
BW12	1665 ^b	52.6	1690 ^a	49.2	1655 ^d	46.4	1660 ^c	52.4
<i>Daily gains at:</i>								
DG4-6	22.1 ^a	0.91	20.2 ^b	1.36	18.9 ^d	0.94	19.5 ^c	1.05
DG6-8	24.1 ^a	1.17	20.8 ^b	1.18	20.5 ^b	1.21	20 ^c	1.35
DG8-10	15.4 ^d	1.12	20.2 ^a	1.13	19.4 ^b	1.16	18.9 ^c	1.29
DG10-12	23.9 ^b	1.35	23.6 ^c	0.92	23.9 ^b	1.4	24.9 ^a	1.56

A=APRI line, M=Moshtohor line, Means within row, not sharing any letter, are significantly different ($P < 0.05$).

For daily body gains, the averages were mostly moderate and ranged from 15.4 to 24.9 g (Table 5). These results were in agreement with Iraqi *et al.* (2008) who recorded an average daily gain of Moshtohor line in Egypt to be 17.5 to 21.2 g/day. In Spain, V line rabbits were higher, being 36.5 g/day for daily gain between weaning and 8 weeks and 37.9 g/day between weaning and 9 weeks of age (Garcia *et al.*, 2000). Youssef *et al.* (2009) reported that APRI line rabbits in Egypt were lower in average daily gain than those of crossed rabbits obtained here (17.0 to 23.2 g/day).

Heritabilities (h^2):

Heritabilities for body weights and gains estimated by VCE6 software is presented in Table 6. Heritabilities estimated by VCE program for body weights were mostly low or moderate and ranging from 0.06 to 0.18, while the estimates for daily body gains ranged from 0.05 to 0.10. The heritabilities obtained here for body weights and gains were nearly similar to those estimates obtained by some studies in Egypt (Youssef, 2004; Youssef *et al.*, 2009), in Nigeria (Akanno and Ibe, 2005), and in Brazil (Ferraz and Eler, 1996). Published estimates of heritability for daily weight gains were higher than those obtained in this experiment (Piles *et al.*, 2004). Khalil and Al-Homidan (2014) reported that the heritabilities were mostly moderate and ranged from 0.19 to 0.27 for body weights and from 0.18 to 0.34 for daily weight gains.

Table 6: Estimates of heritability \pm standard errors (SE) for growth traits estimated by VCE6 softwares

Trait	Number of records	($h^2 \pm SE$ estimated by VCE6)	Trait	Number of records	($h^2 \pm SE$ estimated by VCE6)
<i>Body weights at:</i>			<i>Daily gains at:</i>		
4 weeks	1201	0.10 \pm 0.033			
6 weeks	1164	0.18 \pm 0.035	4-6 weeks	1163	0.08 \pm 0.001
8 weeks	1116	0.17 \pm 0.039	6-8 weeks	1115	0.10 \pm 0.002
10 weeks	1058	0.06 \pm 0.037	8-10 weeks	1046	0.05 \pm 0.003
12 weeks	984	0.08 \pm 0.043	10-12 weeks	979	0.07 \pm 0.002

VCE6= Variance Component Estimation.

Iraqi (2008) estimated the heritability to be 0.05, 0.38 and 0.20 for body weights at 4, 8 and 12 weeks, respectively and 0.23 and 0.19 for daily weight gains at 4-8 and 8-12 weeks, respectively in Sinai Gabali rabbits.

Crossbreeding analysis:

Direct additive effects (G^I):

The generalized least square solutions of G^I and their percentages for body weights and gains given in Table 7 showed that the estimable solutions of G^I were significantly in favour of Moshtohor line rabbits by 13.5 to 48.1g for body weights and 0.5 to 3.7g for daily weight gains, with percentages ranging from 1.4 to 4.3% for body weights and 2.1 to 18.6% for daily weight gains ($P < 0.01$). However, crossbreeding experiments carried out in Egypt indicated that direct additive effects on most post-weaning body weights and gains were significant (Khalil and Afifi, 1991; Youssef *et al.*, 2009; Ibrahim *et al.*, 2008). Khalil and Afifi (1991) reported significant estimates of G^I for body weight at 12 weeks of age. Abdel-Ghany *et al.* (2000) noted that direct additive effects from crossing New Zealand White with Baladi Red or Baladi Black rabbits were consistently in favour of Baladi Red or Baladi Black for post-weaning body weights and gains. In crossing V line and Baladi Red rabbits, Youssef *et al.* (2009) found that the direct additive effects were in favour of V line rabbits reaching 15.0% at 4 weeks and 13.3% at 12 weeks and the effects for daily body gains were significant reaching 35.7% at the interval of 10–12 weeks. In crossing Saudi Gabali with V line rabbits, the direct additive effects were significantly in favour of V line and ranging from 2.7 to 8.1% for body weights and 3.8 to 13.1% for daily weight gains (Khalil and Al-Homidan, 2014). Likewise, Piles *et al.* (2004) in crossing C line and R line rabbits in Spain reported significant estimates of direct additive effects for body weights and

Table 7: Generalized least square solutions and percentages of direct additive effects ($G^I = G^I_A - G^I_M$) and their standard errors (SE) for body weights and gains in crossing APRI and Moshtohor lines

G^I for body weights (BW)					G^I for daily gains (DG)				
Trait	N	G^I solution (units)	SE	G^I as % [†]	Trait	N	G^I solution (units)	SE	G^I as % [†]
BW4	762	-13.5**	0.11	-2.7					
BW6	729	-33.2**	0.13	-4.3	DG4-6	762	-1.7**	0.025	-8.7
BW8	699	-14.8**	0.16	-1.4	DG6-8	697	-3.3**	0.029	-15.9
BW10	672	-35.2**	1.4	-2.6	DG8-10	611	-3.7**	0.032	-18.6
BW12	628	-48.1**	1.2	-2.9	DG10-12	611	-0.5 ^{NS}	0.033	-2.1

[†]Percentage computed as [Estimate of G^I in units / (A+M)/2] x 100

^{††}NS= non-significant, **= $P \leq 0.01$

gaily gains at 32- 60 days. Ouyed and Brun (2008) in crossing Californian with New Zealand White found that the estimates of G^I were in favour of New Zealand White for 63-d body weight.

Maternal additive effect (G^M):

The solutions of maternal effects and their percentages for body weights and gains given in Table 8 showed that the estimable solutions of G^M were significantly in favour of Moshtohor line by 14.2 to 51.8 g for body weights and 0.48 to 3.1g for daily weight gains in weight, with percentages ranging from 2.8 to 5.4% for body weights and 1.8 to 15.0% for daily weight gains. Similarly, crossbreeding experiments carried out in Egypt indicated that maternal effects on most body weights and gains were significant (*e.g.* Abou Khadiga *et al.*, 2008; Ibrahim *et al.*, 2008; Iraqi *et al.*, 2008; Hekil *et al.*, 2011). Abd El-Ghany *et al.* (2000) reported that maternal additive effects on body weights and daily weight gains were significantly in preference of New Zealand White at all ages considered, While Abou Khadiga *et al.* (2008) found that maternal genetic effects were significantly in favor of V line dams for body weights at 8 and 12 weeks of age. Iraqi *et al.* (2008) found that Gabali breed was significantly superior in G^M over V line ($P < 0.01$) for body weights at 8 and 12 weeks of age and daily weight gains at intervals of 4-8 and 8-12 weeks of age.

Direct Heterosis (H^I):

The generalized least square solutions of H^I and their percentages for body weights and gains given in Table 9 indicated that the estimable solutions of direct heterosis were positive and highly significant and ranged from 15.5 to

Table 8: Generalized least square solutions and percentages of maternal effects ($G^M = G^M_A - G^M_M$) and their standard errors (SE) for body weights and gains in crossing APRI and Moshtohor lines

G^M for body weights (BW)					G^M for daily gains (DG)				
Trait	N	G^M solution (units)	SE	G^M as % ⁺	Trait	N	G^M solution (units)	SE	G^M as % ⁺
BW4	762	-14.2**	0.09	-2.8					
BW6	729	-42.4**	0.11	-5.4	DG4-6	762	-1.02*	0.018	-5.2
BW8	699	-37.2**	0.13	-3.5	DG6-8	697	-3.1**	0.024	-15.0
BW10	672	-47.3**	0.8	-3.5	DG8-10	611	-2.9**	0.026	-14.6
BW12	628	-51.8**	1.01	-3.1	DG10-12	611	-0.48 ^{NS}	0.024	-1.8

⁺Percentage computed as [Estimate of G^M in units/ (A+M)/2] x100

⁺⁺NS= Non-significant, *=P<0.05 and **=P<0.01

Table 9: Generalized least square solutions and percentages of heterotic effects ($H^I = H^I_A - H^I_M$) and their standard errors (SE) for body weights and gains in crossing APRI and Moshtohor lines

H^I for body weights (BW)					H^I for daily gains (DG)				
Trait	N	H^I solution (units)	SE	H^I as % ⁺	Trait	N	H^I solution (units)	SE	H^I as % ⁺
BW4	762	15.5**	0.16	3.1					
BW6	729	62.6**	0.19	8.1	DG4-6	762	0.2 ^{NS}	0.026	1.1
BW8	699	87.1**	0.24	8.2	DG6-8	697	2.7**	0.044	13.0
BW10	672	70.5**	1.3	5.2	DG8-10	611	1.6*	0.005	7.8
BW12	628	63.9**	2.1	3.8	DG10-12	611	2.3**	0.049	9.6

⁺Percentage computed as [Estimate of H^I in units/ (A+M)/2] x100

⁺⁺NS= non-significant, *=P<0.05 and **=P<0.01.

87.1g for body weights and from 0.2 to 2.7g for body gains, with percentages ranging from 3.1 to 8.2% for body weights and 1.1 to 13.0% for daily weight gains. Afifi *et al.* (1994) found that heterosis percentages in crossing New Zealand White with Baladi rabbits in Egypt were positive and ranged from 2.7 to 9.5% for post-weaning body weights and daily weight gains. Iraqi *et al.* (2008) found that the estimates of heterosis were always significantly positive by 6.9, 3.6 and 5.4% for body weights at 4, 8 and 12 weeks and 9.7 and 6.1% for daily weight gains at intervals of 4-8 and 8-12 weeks, respectively. Youssef *et al.* (2009) found that direct heterosis were positive and ranged from 4.9 to 16.7% for body weights and 14.4 to 29.5% for daily weight gains. Khalil and Al-Homidan (2014) reported that direct heterosis were significantly positive and ranged from 4.5 to 5.4 % for body weights and from 6.6 to 9.6 % for daily weight gains.

Polymorphic associations between genotypes of GH gene and growth traits:

The generalized least square means for SNP genotypes of *GH* gene in V line, M line, A line and ½A½M cross have shown significant effects on most body weights and gains (Table 10). Similarly, several studies reported significant associations between GH gene and body weights in rabbits (e.g. Fontanesi *et al.*, 2008; Abdel-Kafy *et al.*, 2016; El-Sabrouh and Aggag, 2017).

In V line rabbits, CC genotype was absent and the association of TT and TC genotypes of *GH* gene was significant ($P < 0.05$), since TT genotype was heavier for body weight at 4 weeks than TC genotype (Table 10). However, TT genotype was lower than TC genotype for body weights at 6, 8, 10 and 12 weeks. The TC genotype was positively associated with an increase in body weights at 6, 8, 10 and 12 weeks of age by 32, 9, 57 and 108 g, respectively. For daily gain, the CC genotype was absent and the TT genotype was lower than TC genotype and the difference between SNP genotypes of *GH* gene were significant ($P < 0.05$) for most body gains studied (Table 10). These data was in agreement with El-Aksher *et al.* (2016) who found significant associations ($P < 0.05$) between the genotypes SNPs of progesterone receptor gene (*PGR* gene) and body weights for V line and Sinai Gabali rabbits, Also, El-Sabrouh and Aggag (2017) found significant ($P < 0.05$) associations between growth hormone, insulin-like growth factor 1, myostatin, melanocortin 4 receptor, growth hormone receptor and phosphoglycerate mutase genes and body weights in V line and Alexandria rabbits.

In Moshtohor line rabbits, the differences among genotypes of *GH* gene were significant ($P < 0.05$) and the TC genotype was heavier in body weights at 4, 6, 8, 10 and 12 weeks than CC and TT genotypes (Table 10). The TC genotype was positively associated with an increase in body weights at 4, 8, 10 and 12 weeks of age by 134, 119, 636 and 734g, respectively. The differences among genotypes of *GH* gene in all daily weight gains were significant ($P < 0.05$) and the TT genotype was lower in daily weight gains at 6-8, 8-10 and 10-12 weeks than CC and TC genotypes. The TT genotype was heavier in daily gain at 4-6 weeks than CC and TC genotypes. Lu *et al.* (2011) and Peng *et al.* (2013) reported significant association between myostatin gene (*MSTN*) and growth traits in different genetic groups of rabbits.

The differences between genotypes of *GH* gene in APRI line rabbits were significant ($P < 0.05$) and CC genotype was heavier in body weight at 4 and 10 weeks than TT and TC genotypes, while the TC genotype was heavier in body weights at 6, 8, and 12 weeks than CC and TT genotypes (Table 10). The TC genotype was positively associated with an increase in body weights at 6, 8 and 12 weeks of age by 43, 61 and 305 g, respectively. Significant differences ($P < 0.05$) were recorded between genotypes of *GH* gene in daily

Table 10: Generalized least square means and their standard errors (GLM±SE) for body weights (BW) and daily gains (DG) as affected by SNP genotypes of GH gene in each genetic group separately

Trait	Breed or genetic group	Number of records	Genotypes					
			TT		TC		CC	
			GLM	SE	GLM	SE	GLM	SE
<i>Body weights (g):</i>								
BW4	V line	62	499 ^a	11.6	456 ^b	12.9	--	--
	M line	46	450 ^c	31.6	584 ^a	22.7	498 ^b	30.8
	A line	35	495 ^a	19.4	474 ^b	8.8	496 ^a	21.2
	½A½M	68	494 ^a	11.7	495 ^a	7.4	--	--
BW6	V line	61	770 ^b	19.9	802 ^a	21.5	--	--
	M line	46	795 ^b	30.8	796 ^b	22.3	827 ^a	30.2
	A line	34	707 ^b	26.2	737 ^a	10.9	694 ^c	25.
	½A½M	64	780 ^a	19.2	758 ^b	12.3	--	--
BW8	V line	58	1143 ^b	22.0	1152 ^a	23.9	--	--
	M line	46	1029 ^c	43.4	1148 ^a	31.4	1098 ^b	42.7
	A line	34	1093 ^a	70.6	1095 ^a	28.8	1034 ^c	69.8
	½A½M	60	1043 ^a	24.7	1037 ^b	16.1	--	--
BW10	V line	55	1297 ^b	15.9	1354 ^a	16.9	--	--
	M line	46	1071 ^c	189	1707 ^a	134	1288 ^b	164
	A line	34	1361 ^b	66.2	1271 ^c	27	1399 ^a	65.4
	½A½M	60	1349 ^a	26.9	1261 ^b	17.8	--	--
BW12	V line	50	1696 ^b	24.5	1804 ^a	24.5	--	--
	M line	45	1240 ^c	75	1974 ^a	52.1	1564 ^b	70.8
	A line	29	1673 ^b	94.8	1917 ^a	32.1	1612 ^c	89.1
	½A½M	59	1686 ^a	31.7	1657 ^b	20.7	--	--
<i>Daily gains (g):</i>								
DG4-6	V line	61	18.8 ^b	1.29	25.3 ^a	1.35	--	--
	M line	46	24.6 ^a	2.9	15.1 ^c	2.05	23.5 ^b	2.5
	A line	34	14.7 ^b	1.65	18.6 ^a	0.71	14.1 ^b	1.42
	½A½M	64	20.3 ^a	1.36	18.9 ^b	0.94	--	--
DG6-8	V line	58	28.2 ^a	1.55	20.4 ^b	1.63	--	--
	M line	46	16.7 ^c	3.6	25.1 ^a	2.5	19.3 ^b	3.1
	A line	34	27.3 ^a	4.64	20.1 ^b	2.02	18.8 ^c	4.04
	½A½M	60	18.9 ^b	1.62	20.2 ^a	1.10	--	--
DG8-10	V line	55	9.3 ^b	1.27	20.7 ^a	1.37	--	--
	M line	46	22.2 ^b	13.4	11.3 ^c	10.4	32.8 ^a	12.4
	A line	34	19.3 ^b	2.79	18.1 ^c	1.21	31.4 ^a	2.42
	½A½M	60	16.8 ^b	1.62	22.1 ^a	1.09	--	--
DG10-12	V line	50	29.6 ^a	1.43	21.3 ^b	1.40	--	--
	M line	45	19.3 ^c	3.02	25.5 ^b	2.23	26.9 ^a	2.80
	A line	29	22.6 ^c	5.52	24.0 ^b	1.95	34.9 ^a	4.51
	½A½M	59	31.1 ^a	1.82	22.5 ^b	1.25	--	--

GH=Growth hormone gene; A=APRI line, M=Moshtohor line; letters in the same row indicate significant differences at $P < 0.05$.

weight gains at 6-8 and 10-12 weeks in APRI line. In China, Zhang *et al.* (2012) found significant associations ($P < 0.05$) between growth hormone receptor gene (*GHR*) and body weights in Tianfu Black, Ira and Champagne rabbits. Additionally, Wu *et al.* (2015) reported significant association ($P < 0.05$) between phosphoglycerate mutase gene (*PGAM2*) and growth traits in the same Chinese rabbit breeds.

The differences between genotypes of *GH* gene in $\frac{1}{2}A\frac{1}{2}M$ cross rabbits were significant ($P < 0.05$) in all body weights except body weight at 4 weeks and the CC genotype was absent (Table 10). The TT genotype was heavier than TC genotype in all body weights and this TT genotype was positively associated with an increase of 22, 6, 88 and 29 g in body weights at 6, 8, 10 and 12 weeks of age, respectively. For body gains, the differences between genotypes of *GH* gene in $\frac{1}{2}A\frac{1}{2}M$ rabbits were significant ($P < 0.05$) and the TT genotype was heavier than TC genotype in all body gains, and the CC genotype was absent, while the TC genotype was higher than TT genotype at 8-10 weeks. Abdel-Kafy *et al.* (2016) studied the association between Myostatin (*MSTN*) gene and growth traits in APRI line and found that Allele T at the c.747+34C>T SNP was significantly associated ($P < 0.05$) with increased body weight at 12 weeks of age. Moreover, Othman *et al.* (2015) found that the genotypes of fibroblast growth factor 5 gene (*FGF-5*) were associated significantly ($P < 0.05$) with body weights in different populations of rabbits in Egypt.

CONCLUSIONS

- ❖ Crossing APRI line with Moshtohor line was associated with heterotic effects and could be beneficial to produce rabbits characterized by heavy body weights and gains.
- ❖ Based on direct and maternal effects, APRI line could be used as a sire-group and Moshtohor line as a dam-group in synthesizing new line of rabbits in Egypt to improve growth traits.
- ❖ The significant associations between *GH* gene and growth traits confirmed that this gene could be as a candidate gene and may be used as Marker-assisted selection in genetic improvement programs (MAS) to improve growth performance in rabbits.

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تأثيرات الخلط وإرتباطات التنوع الجزيئي للتراكيب الوراثية لجين هرمون النمو مع صفات النمو في الأرانب

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² قسم تنمية الثروة الحيوانية، كلية الطب البيطري، جامعة بنها - مصر.

أجريت تجربة خلط بسيط بين خطين من خطوط الأرانب المستنبطة حديثاً (خط أبري وخط مشتهر) بهدف تقدير تأثيرات الخلط (الأثر التجمعي المباشر، الأثر الأمي المباشر وقوة الخلط المباشرة) على وزن الجسم بعد الفطام في عمر 4، 6، 8، 10، 12 أسبوع ومعدل الزيادة اليومية في الوزن خلال الفترات من 4 إلى 6، من 6 إلى 10، من 8 إلى 10 ومن 10 إلى 12 أسبوع، وكذلك الكشف عن الارتباطات التنوعية الجزيئية بين التراكيب الوراثية المختلفة لجين هرمون النمو مع صفات وزن الجسم ومعدل الزيادة في الوزن بعد الفطام. استخدمت في هذه الدراسة أربعة مجاميع تربية هي خط أبري، خط مشتهر، خليط الجيل الأول (½ أبري ½ مشتهر) بالإضافة إلى خط الفي لاين الأسباني كعشيرة مرجعية للمقارنة. تم تطبيق تقنية التنوع الجزيئي لأطوال القطع المحدد والمعتمد على تفاعل إنزيم البلمرة المتسلسل باستخدام إنزيم القطع BstUI وذلك لمعرفة نمط التنوع الجزيئي للنيوكليوتيدة المفردة لجين هرمون النمو (TT, TC, CC).

كانت النسب المئوية للأثر التجمعي المباشر معنوية لصالح خط مشتهر 2.7، 4.3، 1.4، 2.6، 2.9% لصفات وزن الجسم عند عمر 4، 6، 8، 10، 12 أسبوع وكانت 8.7، 15.9، 18.6% لصفات معدل الزيادة اليومية في الوزن خلال الفترات من 4 إلى 6، 6 إلى 8، ومن 8 إلى 10 أسابيع علي الترتيب. كانت النسب المئوية للأثر الأمي المباشر أيضاً معنوية لصالح خط مشتهر 2.8، 5.4، 3.5، 3.5، 3.1% لصفات وزن الجسم عند عمر 4، 6، 8، 10، 12 أسبوع علي الترتيب، وتراوحت النسب بين 1.8 إلى 15.0% لصفات معدل الزيادة اليومية في الوزن. كانت النسب المئوية لقوة الخلط موجبة وعالية المعنوية 3.1، 8.1، 8.2، 5.2، 3.8% لصفات وزن الجسم عند عمر 4، 6، 8، 10، 12 أسبوع وكانت نسب قوة الخلط 13، 7.8، 9.6% لصفات معدل الزيادة اليومية في الوزن خلال الفترات من 6 إلى 8، من 8 إلى 10، ومن 10 إلى 12 أسبوع. كانت هناك فروق معنوية بين المتوسطات المحسوبة بطريقة المربعات الصغرى للتراكيب الوراثية المختلفة لجين هرمون النمو لصفات وزن الجسم عند عمر 4، 6، 8، 10، 12 أسبوع في خطوط الفى لاین الأسباني، خط مشتهر، خط أبري، وخليط الجيل الأول (1/2 أبري 1/2 مشتهر). إرتبط التركيب الوراثي TC إرتباطاً معنوياً موجباً مع الزيادة في وزن الجسم في خطوط الفى لاین الأسباني، خط مشتهر، وخط أبري، بينما التركيب الوراثي TT إرتبط بالزيادة في وزن الجسم في خليط الجيل الأول (1/2 أبري 1/2 مشتهر). الإرتباطات المعنوية بين جين هرمون النمو وصفات النمو تؤكد إمكانية استخدام هذا الجين في الانتخاب بمساعدة الواسمات لتحسين أداء النمو في الأرانب.

التوصية: يمكن استخدام جين هرمون النمو كجين فعال مرشح Candidate gene في برامج التحسين الوراثي لتحسين أداء النمو في الأرانب.