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Genetic Evaluation of the Impact of Mating Between Commercial Laying Hens (LSL) with Some Local Egyptian Strains Chicken and its Effects on Some Productive and Physiological Parameters

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ABSTRACT

The objective of this study is to investigate the genetic similarity between hybrids and commercial strain chickens using random amplyphysim polymorphisem DNA (RAPD-PCR) marker, as well, to compare some productive and physiological traits. Locally developed strains of chickens (Silver Montazah (SM), Inshase (IN) and Matrouh (Mt)) with one commercial strain Lohman Selected Leghorn (LSL) were mated to produce the three way-crosses Mt×LSL×SM and Mt×LSL×IN. Results revealed that, Pullets of LSL strain matured earlier (151.3 day) than three way-crosses Mt×LSL×SM and Mt×LSL×IN (154.67 and 160.7 day), respectively. Crossbred Mt×LSL×IN had superior annual egg production (172 eggs) than Mt×LSL×SM (154 eggs). The LSL strain had the heaviest egg weight and egg mass means during the 52 weeks of production compared to the tow three way-crosses. Plasma Estrodiol 17-β (E_2) concentration of LSL strain was higher compared to the three local strains chickens. Increasing both of plasma E_2 and E_2/P_4 ratios of LSL strain and crossbred chickens are correlated with higher egg production traits. Positive heterosis percentage for body weight at sexual maturity, annual egg number and annual egg mass of the 3-way cross were found while the age at sexual maturity, egg production traits during the 1st 90 days and annual egg weight had negative heterosis percentage. The genetic similarity among chicken hybrid and LSL parent was estimated as 37% between the 3-way cross (Mt×LSL×SM) and LSL and 33% between the 3-way cross (Mt×LSL×IN) and LSL strain based on RAPD-PCR. Generally, The three-way crosses improved age at sexual maturity and annual egg number, also, increased plasma E_2 and P_4 hormones concentration compared to the pure strain. The result of DNA analysis indicated that genetic similarity may coincide with percent heterosis and this result agreed with the results of economic traits and hormones analysis.

Key words: Chickens, heterosis, RAPD-PCR markers, three-way cross

INTRODUCTION

Heterosis is the phenotypic expression of a complex phenomenon which may involve several types of genetic effects like dominance and epistasis. In animal breeding, basic quantitative genetics theory indicates that heterosis should be proportional to differences in gene frequency between

populations (Hill and Mackay, 2004), so it is commonly used for planning crosses. The heterosis for egg production reported in literature is highly variable, as it depends on the nature and degree of differences among strains but it is often around 10% or greater (Fairfull, 1990).

Spontaneous ovulations are induced by preovulatory surges of Luteinizing Hormone (LH) and progesterone during ovulatory cycles in birds but estradiol-17-β levels are relatively constant. Mashalay *et al.* (1979) and Su *et al.* (1996) showed that there was a significant positive correlation between serum progesterone concentration and egg production in control birds and those received estradiol17-β or progesterone. However, Khalifa *et al.* (1983) reported that estradiol treatment was found to improve egg number or egg mass significantly. Increase in egg production by the estradiol treatment was confirmed by higher clutch size. This improvement in egg production by estradiol could be explained by the physiological effect of estrogen upon the ovary and oviduct through causing their activation and enhancing ovulatory process. On the other hand, egg weight was increased by estradiol treatment over control but not significantly. Egg production is associated with intensive metabolic activities (Leeson and Summers, 1990) and increasing estradiol hormones secretion (Etches, 1993). Hamdy *et al.* (2002) reported that, egg mass was significantly and positivly correlated with plasma concentration of estradiol, progesterone and luteinizing hormones. DNA fingerprint has been shown a useful tool for the assessment of genetic distances between genetic groups of poultry (Kuhnlein *et al.*, 1989; Dunnington *et al.*, 1991; Haberfeld *et al.*, 1992). According to Howard and Moore (1984), there are various well-developed strains of poultry that are used commercially. Ali and Ahmed (2001) proved that there were molecular differences between Egyptian chicken strains. However, Aly and Abdel-Rahman (2010) reported that crossing of the developed local hens has improved the body weight of their progeny at marketing age. Therefore, they attributed the ability of RAPD-PCR for establishing the association between genetic similarity to band sharing and heterosis.

The objectives of this study were to investigate the genetic similarity between hybrids and commercial strain chickens using Random amplyphysim polymorphisem DNA (RAPD-PCR) markers, as well, to compare some productive and physiological traits.

MATERIALS AND METHODS

The present study was carried out at El-Sabhia Poultry Research Station, Animal Production Research Institute, Agricultural Research Center, at the Ministry of Agriculture, Egypt. The analysis of RAPD-PCR technique was determined at City for Scientific Research and Biotechnology Application, Borg EL-Arab, Egypt. Three developed local strains of chicken (Matrouh, Inshase and Silver Montazah) and one commercial strain (Lohman Selected Leghorn (LSL)) were used to produce the three-way crosses. In the first generation, Matrouh was mated as a sire line with Silver Montazah (SM), Inshas (IN) or Lohman Selected Leghorn (LSL) as a dam lines to produce their two-way crosses (Mt×SM, Mt×LSL and Mt×SM). Artificial insemination had been applied by assigning 4 females to each male for this generation. Then, in the second generation, both of the two-way crosses were crossed to produce the three-way crosses as follow: 10 males of MT×SM were mated to 100 females of MT×LSL to produce the 3-way crosses of MT×LSL×SM. moreover, 10 males of MT×IN were mated to 100 females of MT×LSL to produce the 3-way crosses of MT×LSL×IN. Natural mating was carried out using 10 family pens to produce the three way crosses (each pen contained 1 male per 10 females). All experimental birds were maintained under the same conditions as much as possible.

Chicks of each genotype were wing-banded, weighed after hatching and brooded on floor brooders during the first eight weeks of age. At eight weeks of age, the chicks were sexed, weighed and moved to the rearing house. At about 18 weeks of age, the females were assigned to individual laying cages. Age and weight at sexual maturity were individually recorded. Egg number and egg weight during the first 90 days of production were recorded daily. After 90 days of egg production, hens were removed to the breeding pens and hen day egg production and egg weight were recorded. Feed and water were provided *ad libitum*.

Blood biochemical characteristics: At 40 week of age, blood samples were withdrawn from wing vein from 10 birds chosen randomly of each pure strain and of the 3-way crosses. Plasma was obtained by centrifuging the blood at 3000 rpm for 20 min then stored at -20°C for later analysis. Plasma (E_2) pg mL^{-1} and progesterone (P_4) ng mL^{-1} concentration were determined according to the method of Miller (1988) and Canez *et al.* (1992). Estrogen and progesterone ratio (E_2/P_4) was calculated. Plasma cholesterol, high density lipoprotein (HDL mg dL^{-1}) and low density lipoprotein (LDL mg dL^{-1}) were determined by method of Watson (1960) and total lipids by method of Frings *et al.* (1972). Plasma calcium (Ca mg dL^{-1}) was determined according to method of Tietz (1986).

DNA extraction: For molecular analysis, blood samples were taken from five females chosen randomly at 40 weeks of age of each of the two three-way crosses and LSL strain by heparinized syringes via., cardiac puncture and then stored at -20°C until DNA extraction.

Total DNA was extracted according to Sharma *et al.* (2000). The 700 μL of lyses buffer (10 mM Tris-HCl, 100 mM NaCl, 1 mM EDTA, pH 8.0, 0.5% SDS) and 60 μg of proteinase K (20 mg mL^{-1}) were added to 100 μL thawed blood. The mixture was vortexed and incubated at 37°C overnight. DNA was extracted by equal volumes of phenol-chloroform-isoamylalcohol (25:24:1) and chloroform-isoamylalcohol (24:1), successively. DNA was precipitated by adding two equal volumes of chilled ethanol (95%). The pellet was washed with 70% ethanol, air-dried and subsequently dissolved in an appropriate volume of double distillated water (ddH2O).

RAPD analysis: Six random primers were used to RAPD analysis (Table 1). The reaction mixture was carried out in total volume 25 μL containing 10 ng DNA of each sample, 10x buffer, with MgCl, 0.2 mM each of dATP, dTTP, dCTP, dGTP, 25 pmol primer and 0.5 unit of *Taq* DNA polymerase (Promega, Germany). PCR amplification was performed on thermocycler (Eppendorf 2231, Hamburg, Germany) using the following PCR program, 95°C for 5 min; 40 cycles of 95°C for 1 min, annealing at 30°C for 1 min and extension at 72°C for 1 min. A final extension step at 72°C for 10 min PCR products were separated on agarose gel electrophoresis using 1.5% and then visualized

Table 1: List of the random primers and their nucleotide sequence

Primers	Sequence 5´→3´
OPA 01	CAGGCCCTTC
OPA 02	TGCCGAGCTG
OPA 04	AATCGGGCTG
OPA 08	GTGACGTAGG
OPA 13	CAGCACCCAC
OPX 15	CAGACAAGCC

on gel documentation system. The amplified fragments were scored as 1 for presence and 0 for absence of band and the data were analyzed for clustering using the formula of (Nei and Li, 1979). A similarity coefficient was used for cluster analysis following the Unweighed Pair Grouping Method of Averages Method (UPGMA).

Statistical analysis: The data was statistically analyzed to test the significance of the difference between means of genotypes or genetic groups (three way crossbred) (SAS, 2000). Duncan's multiple range test was used to compare every two means (Duncan, 1955) of the different studied traits.

The following model was used:

$$Y_{ijk} = \mu + B_i + L_j + e_{ijk}$$

Where:
Y_{ijk} = Observation of the ijk pullet
μ = Overall mean
B_i = Fixed effect of genetic group
L_j = Fixed effect of genotype (pure or cross) within the ith breeding group
e_{ijk} = Remainder error

Heterosis percentage (H %) based on mid Parent (MP) was determined according to equations given by Lasley (1978):

$$H\ (\%) = F1\text{-}MP \times 100\ MP$$

Where:
H (%) = Heterosis percentage
F1 = Mean of the three way cross
MP = Mid parent

RESULTS AND DISCUSSION

Means and standard error of body weight (BWSM) and age (ASM) at sexual maturity for the pure strains, 2-way crosses and 3-way crosses are present in Table 2. No significant differences were found between the four pure strains (LSL, Mt, IN and SM) on BWSM, though the LSL pullets had the heaviest BWSM (1493.13 g) compared to the developed local strains. On the other hand, no significant effect was found due to the crossing between Mt and LSL (to produce the two-way crosses) on BWSM which produced pullets with the same BWSM. Whereas, crossing between the local pure strains resulted in increase of BWSM in both Mt×IN and Mt×SM pullets and this increase was significant. These results are in agreement with Ghanem *et al.* (2012a) who reported that LB commercial strain had significantly the heaviest BWSM when compared with Mandarah and SM strains. With regard to the effect of crossing in 3-way cross, Body Weight at Sexual Maturity (BWSM) in both Mt×LSL×IN and Mt×LSL×SM appeared to be significantly higher compared to the pure strains and this increase averaged 1571.02 g vs. 1473.42 g in 3-way crosses and pure strain, respectively.

Age at the first egg (ASM) was significantly lowest in the LSL commercial strain compared to the other three local strains and this decrease was approximately 46 days. On the other side, within

Table 2: Means and standard errors of body weight (BWSM) and age (ASM) at sexual maturity for pure stains (2-way and 3-way crosses)

Genotypes	No. of hens	BWSM	ASM
LSL	74	1493.13±14.9^{bc}	144.56±0.93^{e}
Mt	44	1456.09±27.71^{c}	187.41±0.59^{b}
IN	49	1481.24±74.14^{bc}	193.76±1.52^{a}
SM	56	1458.25±23.36^{c}	187.63±1.12^{b}
Overall mean of pure strains	223	1473.42±12.7^{B}	171.30±1.64^{A}
Mt×LSL	71	1488.62±41.7^{bc}	182.52±0.22^{b}
Mt×IN	73	1535.84±33.19^{abc}	166.06±2.08^{c}
Mt×SM	75	1620.08±65.50^{a}	181.31±4.17^{b}
Overall mean of 2-way crosses	219	1537.43±24.82^{A}	174.43±1.62^{A}
Mt×LSL×IN	65	1550.44±32.81^{abc}	160.74±3.04^{c}
Mt×LSL×SM	70	1587.75±33.09^{ab}	154.67±2.27^{d}
Overall mean of 3-way crosses	135	1571.02±23.40^{A}	157.39±1.87^{B}
Significance of genotype		**	**
Significance of genetic group		**	**

^{a,b,c,A,B}Different letters in every column are significantly different, **Significant at $p \leq 0.01$. LSL: Lohman selected Leghorn, Mt: Matrouh, IN: Inshase, SM: Silver montazah

the developed local strain (Mt, IN and SM) it could be noticed that IN had the highest ASM compared to the other two strains which were identical. Crossing between Mt strain as a sire and the other three strains (LSL, IN and SM) as a dame resulted in reducing the Age at Sexual Maturity (ASM) compared to the developed local strains but this was still significantly higher than that obtained by LSL strain. Though, IN strain had the highest ASM, the cross between Mt and IN strains to produce the two-way crosses revealed a decrease in this point compared to the basic strains. Using the crossing to produce the three-way crosses (Mt×LSL×IN and Mt×LSL×SM) resulted in reducing the Age at Sexual Maturity (ASM) compared to the developed local strain or the 2-way crosses and this effect was significant but that was still significantly higher ($p \leq 0.01$) than the LSL strain. The overall means of ASM were 171.3, 174.43 and 157.39 days of pure strain, 2-way crosses and 3-way crosses, respectively. This result indicated that the 3-way crosses decreased the age at sexual maturity by 17.04 day than 2-way crosses and by 13.91 day than the pure strains. Similar results were reported by Nawar and El-Deen (2000) and Ghanem *et al.* (2012b).

Within three-way crosses, Mt×LSL×SM pullets matured significantly earlier by about 6 days than the Mt×LSL×IN cross. It may be necessary to use LSL strain to produce three-way crosses relieved the deleterious effect that happened due to two-way crosses produced by crossing between developed local strains. Differences in sexual maturity may be due to the genetic makeup, these results agreed with those reported by Iraqi *et al.* (2007) and Ghanem *et al.* (2012a).

For egg production traits during the 1st 90 days (EN90), LSL commercial strain laid number of eggs with egg weight (EN90 and EW90) significantly higher than the developed local strains (Table 3). Increase egg number and egg weight in LSL were reflected on the increase of egg mass during the 1st 90 days of laying (EM90) that was significantly higher in LSL strain than the other local strain. On the other hand, Mt strain had the lowest means of EN90 and EM90 compared to the LSL, IN or SM strain. This result was in agreement with Iraqi *et al.* (2007), Nawar (2009) and Ghanem *et al.* (2013) who reported that commercial strain had the highest egg production traits when compared with the developed strains.

The data also revealed that crossing between developed local strain to produce the 2-way crosses (Mt×IN or Mt×SM) resulted in a deleterious effect on EN90 and EM90 which was significantly lower compared to the basic strains. Whereas, crossing between Mt×LSL showed a significant increase ($p<0.01$) in EN90 which surpassed LSL. While Mt×LSL cross had lower EW90 and EM90 means compared to LSL strain. Of the 3-way crosses, hens of Mt×LSL×IN cross and Mt×LSL×SM cross laid number of eggs during the 1st 90 days of laying nearly similar to the local pure strains but it was significantly lower than that of LSL. EW90 and EM90 had the same trend.

Data of egg number, egg weight and egg mass during 365 days of hen's production (EN365, EW365 and EM365) in the 2-way crosses had the same trend of EN90, EW90 and EM90 (Table 4). The data revealed that crossing between crosses (Mt×IN or Mt×SM) resulted in

Table 3: Means and standard errors of egg number (EN90), egg weight (EW90) and egg mass (EM90) during the 1st 90 days of laying for pure stains (2-way and 3-way crosses)

Genotypes	No. of hens	EN90	EW90	EM90
LSL	74	51.10±0.98^{b}	59.62±0.37^{a}	3042.61±58.50^{a}
Mt	44	41.83±1.29^{d}	47.14±0.41^{e}	1963.31±50.23^{e}
IN	49	48.82±0.48^{bc}	47.10±0.39^{e}	2288.54±18.80^{b}
SM	56	44.22±1.88^{dc}	45.85±0.17^{cd}	2001.08±85.36^{bc}
Overall mean of pure strains	223	48.98±0.77^{A}	55.03±0.54^{A}	2704.89±54.41^{A}
Mt×LSL	71	57.84±1.78^{a}	49.94±0.81^{b}	2883.48±91.15^{a}
Mt×IN	73	29.08±3.13^{e}	44.58±0.44^{d}	1270.80±81.30^{d}
Mt×SM	75	28.42±1.76^{e}	46.74±0.84^{cd}	1359.93±147.29^{d}
Overall mean of 2-way crosses	219	37.43±2.08^{C}	46.64±0.47^{B}	1775.56±108.33^{C}
Mt×LSL×IN	65	46.51±1.34^{cd}	49.56±0.57^{b}	2302.78±69.70^{b}
Mt×LSL×SM	70	45.02±1.24^{dc}	45.59±0.82^{cd}	2068.17±73.90^{bc}
Overall mean of 3-way crosses	135	45.69±0.91^{B}	47.37±0.56^{B}	2173.34±52.60^{B}
Significance of genotype		**	**	**
Significance of genetic group		**	**	**

^{a,b,c,d,e,A,B,C}Different letters in every column are significantly different, **Significant at $p\leq0.01$, LSL: Lohman selected Leghorn, Mt: Matrouh, IN: Inshase, SM: Silver montazah

Table 4: Means and standard errors of egg number (EN365), egg weight (EW365) and egg mass (EM365) during annual egg production of laying for pure stains (2-way and 3-way crosses)

Genotypes	No. of hens	EN365	EW365	EM365
LSL	74	169.99±2.78^{a}	63.10±0.36^{a}	10710.20±167.46^{a}
Mt	44	107.70±2.35^{d}	53.90±0.18^{de}	5799.59±121.91^{f}
IN	49	153.54±2.48^{b}	56.36±0.06^{b}	8652.51±138.25^{cd}
SM	56	109.20±2.06^{d}	53.42±0.15^{e}	5832.95±112.10^{f}
Overall mean of pure strains	223	138.70±2.26^{B}	57.36±0.31^{A}	8054.25±161.74^{B}
Mt×LSL	71	178.05±7.16^{a}	54.69±0.15^{cd}	9755.61±416.60^{b}
Mt×IN	73	130.77±2.14^{c}	55.01±0.18^{c}	7187.48±125.37^{e}
Mt×SM	75	115.44±3.09^{d}	54.79±0.16^{cd}	6333.86±171.25^{f}
Overall mean of 2-way crosses	219	138.38±4.38^{B}	54.80±0.08^{B}	7590.74±244.05^{C}
Mt×LSL×IN	65	172.03±3.36^{a}	52.14±0.51^{f}	8964.60±189.23^{c}
Mt×LSL×SM	70	155.23±3.34^{b}	53.59±0.36^{e}	8322.30±189.51^{d}
Overall mean of 3-way crosses	135	162.76±2.53^{A}	52.94±0.31^{C}	8610.23±138.23^{A}
Significance of genotype		**	**	**
Significance of genetic group		**	**	**

^{a,b,c,d,e,f,A,B,C}Different letters in every column are significantly different, **Significant at $p\leq0.01$, LSL: Lohman selected Leghorn, Mt: Matrouh, IN: Inshase, SM: Silver montazah

deleterious effect on productive traits means which was significant. While, Mt×LSL cross showed the highest means values ($p \leq 0.01$) of EN365 (178.05 eggs) and EM365 (9755.61 g) compared to the other crosses (Table 4), this result may be due to the genetic make up of each of Mt (Mahmoud *et al*., 1974) and LSL strain which Leghorn strain was the common parent for each strains.

For 3-way crosses, Mt×LSL×IN cross had significantly higher ($p \leq 0.01$) EN365 than that of Mt×LSL×SM cross (172.03 vs. 155.23, respectively) (Table 4). The same trend was found for egg mass (EM365). While, for annual egg weight, Mt×LSL×SM cross had significantly ($p \leq 0.01$) heavier egg (53.59 g) compared to Mt×LSL×IN cross (52.14 g). This result may be due to the negative genetic correlation between egg number and egg weight (Fairfull, 1990). These results indicate that egg mass may be due to the increased egg number. Moreover, epistasis effects may be control the inheritance of egg number and egg mass. The same conclusion was cited by Wei and van der Werf (1994) and El-Ghar *et al*. (2010). Generally, intromission LSL blood in 2-way crosses (Mt×LSL) or in both 3-way crosses resulted in a slightly increase in EN90 and EN365 but egg weight and egg mass were significantly lower compared to the LSL pure strain.

Results of E_2, P_4 hormones concentrations and E_2/P_4 ratio of pure and crossbreed chickens are shown in Table 5. With respect to pure strain, there were no significant differences between three developed local strains in plasma E_2 and P_4 levels, whereas, the levels of these hormones were highly significant increase in LSL commercial strain compared to the three local strain. Increasing E_2 and P_4 hormone levels which found in LSL strain was reflected on the levels of them in the 3-way cross. The 3-way crosses (Mt×LSL×IN and Mt×LSL×SM) showed E_2 level slightly decrease compared to the LSL value and the differences were non significant. On the other hand, E_2 level in both the 3-way crosses was significantly higher than that of developed local strain. Of the P_4 level, 3-way crosses had higher P_4 means compared to the four pure strain and the differences were significant with the three developed local strains. Increasing plasma E_2 and P_4 of LSL strain and crossbreed chickens are correlated with higher egg production traits, whereas, the EN, EW and EM during the two periods studies of LSL crossbreeds were significantly higher than pure strains. There was a relationship between plasma E_2 concentration and egg production traits which were studied, whereas, the egg production, egg weight and egg mass increased in the three crossbreeds with increasing plasma E_2 concentration compared to local pure strains. Several investigators found

Table 5: Means and standard errors of progesterone (P_4), estrogen (E_2) hormones and estrogen and progesterone ratio (E_2/P_4) for pure stains and 3-way cross

Genotypes	E_2	P_4	E_2/P_4
LSL	250.77±52.92^{a}	1.567±0.05^{ab}	1.19±0.230^{ab}
Mt	114.39±16.43^{c}	1.408±0.02^{b}	1.30±0.330^{ab}
IN	113.76±15.59^{c}	1.310±0.01^{b}	1.61±0.340^{a}
SM	131.31±5.930^{bc}	1.446±0.08^{b}	0.83±0.130^{b}
Overall mean of pure strains	140.71±12.73^{B}	1.420±0.02^{B}	1.24±0.190
Mt×LSL×IN	216.58±35.86^{a}	2.094±0.32^{a}	0.87±0.136^{b}
Mt×LSL×SM	239.49±49.82^{a}	2.087±0.21^{a}	0.96±0.090^{b}
Overall mean of 3-way crosses	228.03±29.91^{A}	2.091±0.18^{A}	0.92±0.120
Significance of genotype	**	**	*
Significance of genetic group	**	**	ns

^{a,b,c,A,B}Different letters in every column are significantly different, **,*Significant at $p \leq 0.01$ and $p \leq 0.05$, respectively, ns: Non significant, LSL: Lohman selected Leghorn, Mt: Matrouh, IN: Inshase, SM: Silver montazah

that E_2 improved hens' egg production (Wilson and Sharp, 1976; Sturkie, 1976). Khalifa *et al.* (1983) concluded that, the improvement of hens' egg production and egg weight with E_2 are due to the physiological effect of E_2 on the ovary and oviduct. Hamdy *et al.* (2002) reported that, egg mass was significantly and positively correlated with plasma concentration of E_2.

There was no-significant difference in plasma total lipids concentration among the four pure strains or the 3-way crosses with increasing its level in the local strains than the LSL and crossed hens (Table 6). Decreasing blood total lipids level was correlated with increasing egg production in LSL and 3-way crosses, where, the fat is withdrawn from the blood to the liver to provide the formation of the yolk lipids.

Data of plasma total cholesterol showed a highly significant increase in the cholesterol level with LSL and Mt pure strain compared to IN and SM while, SM had the lowest cholesterol means. With regard to the 3-way crosses, the Mt×LSL×IN cross had the highest cholesterol means than the Mt×LSL×SM cross which was significant while this level was similar to LSL and Mt strains. Plasma low density lipoprotein concentration had the same trend of the total cholesterol. Though, IN and SM had the lowest total cholesterol means (147.48 and 120.42, respectively), plasma High Density Lipoprotein (HDL) concentration significantly increased compared to the other pure strain or the 3-way crosses and LSL commercial strain had the lowest mean in this trait.

No significant differences were found among the four pure strains in plasma calcium concentration or between the pure strains and the 3-way crosses while observing that plasma calcium level was slightly higher in 3-way crossed hens than all pure strains.

Heterosis percentage: Heterosis percentage (H%) and mean of mid parents of 3-way crosses are presented in Table 7. It showed that the 3-way crosses Mt×LSL×SM realized higher H% for BWSM, ASM, EN365 and EM 365 days than the other one (8.07-10.7%, 20.37 and 11.75%, respectively). Positive H% for BWSM, EN365 and EM365 of the three-way crosses were found while, the ASM, EN90, EW90, EM90 and EW 365 traits had negative H% for 3-way crosses. These results indicated that crossbreeding increased BWSM by 4.99 and 8.07% for Mt×LSL×IN and Mt×LSL×SM, respectively. In addition, it increased annual egg number by 19.68 and 6.88% for Mt×LSL×IN, respectively and egg mass by 20.37 and 11.75% for Mt×LSL×SM, respectively. On the other hand, ASM decreased by 8.25 and 10.7% for both of Mt×LSL×IN and Mt×LSL×SM, respectively.

Table 6: Means and standard errors of plasma total lipids (TL), total cholesterol (Ch), low density lipoprotein (LDL), high density lipoprotein (HDL) and calcium (Ca) concentrations for pure stains and 3-way cross

Genotypes	TL ($g\ dL^{-1}$)	Ch	HDL	LDL	Ca
		($mg\ dL^{-1}$)			
LSL	3.80±0.27	179.63±9.35^{a}	16.22±1.35^{c}	163.41±9.16^{a}	9.71±0.49
Mt	4.02±0.25	177.79±8.38^{a}	20.81±1.64^{abc}	156.98±8.28^{ab}	9.73±0.94
IN	3.95±0.17	147.48±9.91^{bc}	24.75±2.32^{ab}	122.73±9.63^{c}	9.86±1.17
SM	4.10±0.11	120.42±8.24^{c}	26.85±2.51^{a}	93.57±8.77^{d}	9.72±0.76
Overall mean of pure strains	3.99±0.18	157.95±5.61	22.15±1.10	135.80±6.05	9.98±0.44
Mt×LSL×IN	3.50±0.21	176.85±12.38^{a}	19.92±1.79^{bc}	156.93±12.95^{ab}	10.17±0.39
Mt×LSL×SM	3.75±0.26	148.45±13.81^{bc}	18.39±2.81^{c}	130.06±12.52^{bc}	10.08±0.58
Overall mean of 3-way crosses	3.62±0.21	164.43±9.62	19.25±1.54	145.18±9.47	10.13±0.34
Significance of genotype	ns	**	**	**	ns
Significance of genetic group	ns	ns	ns	ns	ns

$^{a..b.c.d}$Different letters in every column are significantly different, **,*Significant at $p \leq 0.01$ and $p \leq 0.05$, respectively, ns: Non significant, LSL: Lohman selected leghorn, Mt: Matrouh, IN: Inshase, SM: Silver montazah

However, decreased EN90, EW90, EM90 and EW365 by 1.57, 3.37, 5.29 and 9.78 for Mt×LSL×IN cross, the corresponding values were 1.53, 10.38, 11.45 and 5.67 for Mt×LSL×SM. The present data showed that the effect of dominance and overdominance of genes (non additive effects) was clear on these traits. The same conclusion was observed by Fairfull *et al.* (1987) and Cheverud and Routman (1995).

DNA fingerprint using RAPD-PCR: The six arbitrary primers used for PCR amplification of the genomic DNA showed bands in the molecular weight ranging from 200-1500 bp (Fig. 1a-f). The results revealed that the samples of both Mt×LSL×IN and Mt×LSL×SM gave the same band pattern with the primers 2, 5 (Fig. 1b, e). The amplification profiles with the primers 1 and 6 showed highly polymorphic profile in the samples of both Mt×LSL×IN and Mt×LSL×SM crosses (Fig. 1a, f). Aphylogenetic tree was generated from RAPD pattern of the three samples (Fig. 2). The samples Mt×LSL×IN and Mt×LSL×SM are grouped in the same cluster while the sample LSL in the separate cluster. The genetic similarity was 37% between LSL and Mt×LSL×IN cross and 33% between LSL and Mt×LSL×SM (Table 8). These results were agreement with the results present in Table 4 which indicated that Mt×LSL×SM cross produced less annual egg number (155.23 egg) than LSL (169.99 egg) and Mt×LSL×IN cross (172.03). Also, the results presented in Fig. 1a, b revealed that there are genetic variations between LSL strain and the 3-way crosses. These results may be due to the genetic make up for LSL and the 3-way crosses.

The RAPD analysis has been used for constructing parsimony tree among the two 3-way crosses and LSL strain to the progeny of Mt×LSL×SM (Fig. 1b) while the other crossbred was very different (Zhang *et al.*, 2004; Nowzari *et al.*, 2005). This method has been used for constructing trees in other organisms such as farm animals: Cattle, buffalo, goat and sheep (Rao *et al.*, 1996; Ali *et al.*, 2003).

Table 7: Heterosis percentage for some egg production traits in the three-way crosses

3-way crosses	BWSM	ASM	EN90	EW90	EM90	EN365	EW365	EM365
Mt×LSL×IN								
Mean	1550.44	160.74	46.51	49.56	2302.78	172.03	52.14	8964.60
Mid parent	1476.82	175.20	47.25	51.29	2431.42	143.7	57.79	8387.43
Heterosis	4.99	-8.25	-1.57	-3.37	-5.29	19.68	-9.78	6.88
Mt×LSL×SM								
Mean	1587.75	154.67	45.02	45.59	2068.17	155.23	53.59	8322.30
Mid parent	1469.16	173.20	45.72	50.87	2335.67	128.96	56.81	7447.58
Heterosis	8.07	-10.70	-1.53	-10.38	-11.45	20.37	-5.67	11.75

BWSM: Body weight at sexual maturity, ASM: Age at sexual maturity, EN90: Egg number during the 1st 90 days of laying, EW90: Egg weight during the 1st 90 days of laying, EM90: Egg mass during the 1st 90 days of laying, EN365: Egg number during annual egg production, EW365: Egg weight during annual egg production, EM365: Egg mass during annual egg production, LSL: Lohman selected Leghorn, Mt: Matrouh, IN: Inshase, SM: Silver montazah

Table 8: Genetic similarities percentage estimated for each primer between 3-way cross and LSL strain based on RAPD data

Genotype	LSL	Mt×LSL×IN	Mt×LSL×SM
LSL	0	37	33
Mt×LSL×IN	37	0	14
Mt×LSL×SM	33	14	0

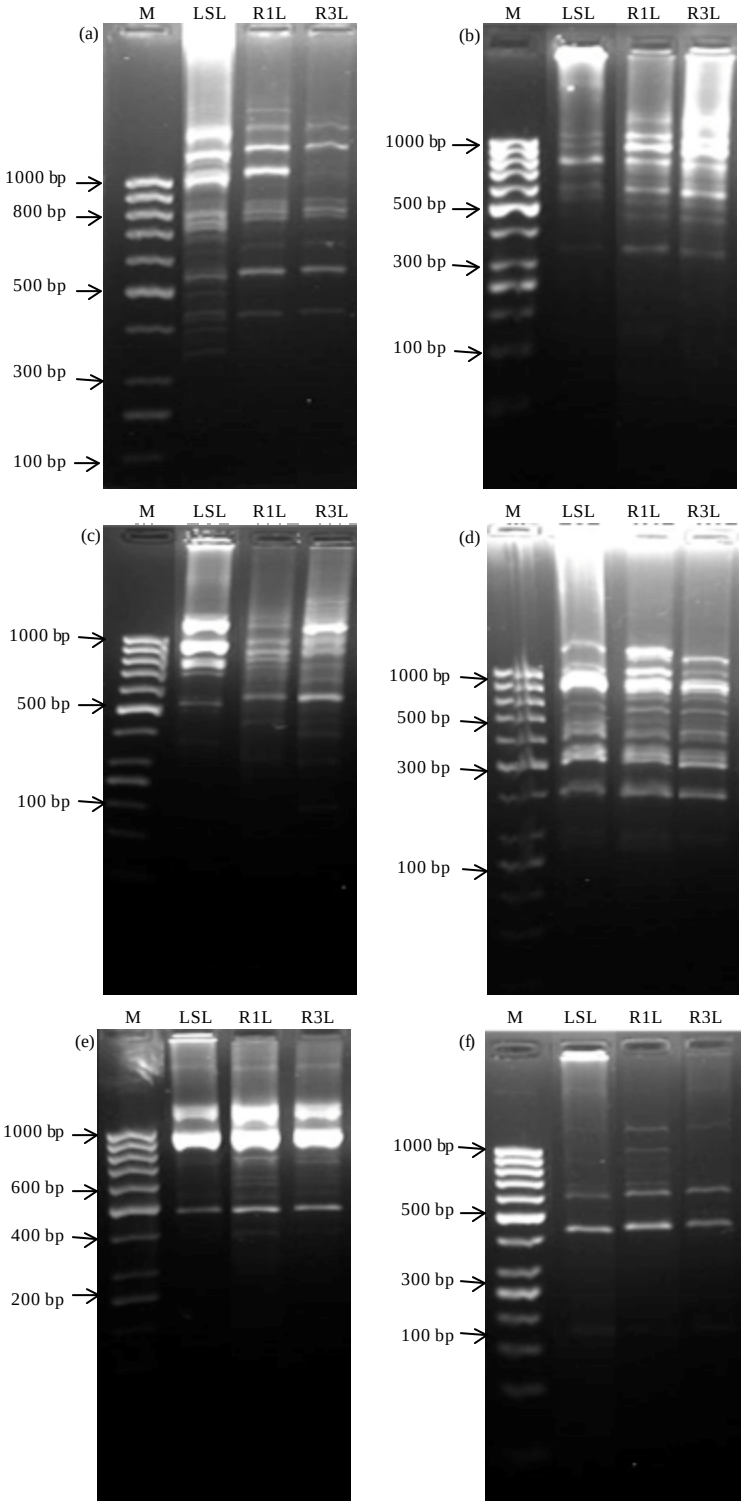

Fig. 1(a-f): RAPD fragment amplified from genomic DNA of LSL strain and the 3-way crosses by primer, Lane M: 50 bp DNA laddes marker, Lane 2: LSL strain, Lane 5: (Mt×LSL×IN) (R1L) and Lane 7: (Mt×LSL×SM) (R3L)

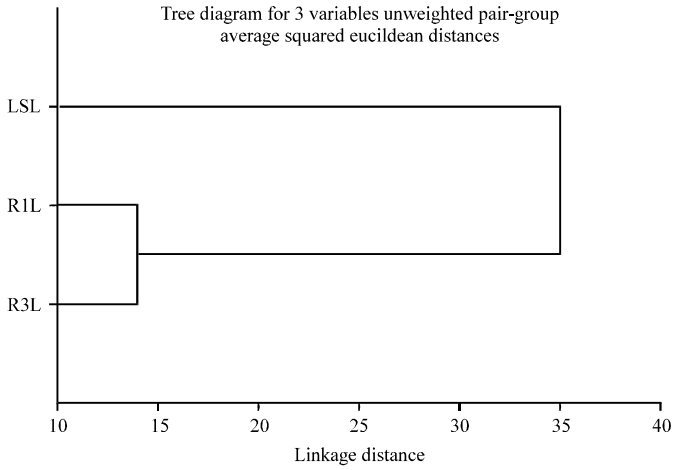

Fig. 2: Dendrograme constructed on the basis of RAPD profile for genomic DNA of LSL strain and the 3-way crosses using six RAPD primers. R1L = Mt×LSL×IN, R3L = Mt×LSL×SM

The results in the present study demonstrated the usefulness of RAPD approach for detecting genetic similarity and/or polymorphisms among 3-way crosses and LSL strain. The majority of arbitrary primers used gave distinctly reproducible patterns in all the breeds' studies. Thus, the RAPD profile generated for LSL strain and 3-way crosses can be effectively used as a supporting marker for taxonomic identification as taxonomic relationship of strains. In taxonomic and systematic molecular, strains relationship markers could be a tool for strains verification and in establishing the status of systematic organisms and their evolution. The obtained results are in agreement with the findings of several previous reports (Sharma *et al*., 2001; Ali *et al*., 2002; Aly and Abdel-Rahman, 2010). This result is supporting such association between DNA fingerprinting similarity and heterosis which has been published before in chickens (Elkomy *et al*., 2007; Aly and Abdel-Rahman, 2010; Ghanem *et al*., 2012b).

CONCLUSION

Generally, the three-way crosses improved age at sexual maturity and annual egg number. It also, increased plasma E_2 and P_4 hormones concentration compared to the pure strain. Homezygosity in DNA of high parent LSL strain and crosses reflects the absence of heterosis in the 3-way cross and high parent.

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