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Quantitative Trait Loci Associated with Egg Traits in F_2 Intercross between Golden Montazah and White Leghorn Chickens

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Abstract

Quantitative trait loci (QTL) affecting age at first egg (AFE), weight at first egg (WFE), 120-days of egg number (EN), egg weight (EW), Hugh unit (HU) and egg shell strength (ESS) were identified in F_2 intercross population produced by crossing males of Golden Montazah (M) with females of White Leghorn (L). Phenotypic data for egg traits of 4131 hens were analyzed using multi-traits animal model. For QTL analysis, 1011 hens of F_2 were genotyped using 45 genetic markers in nine autosomal linkage groups and Z chromosome and the mixed model including the fixed effects of hatch along with the additive and dominance effects of QTL as random effects was used. The total map length was 1949 cM and a total of 15 significant QTL were detected for egg traits and these QTL were distributed over four distinct regions on five chromosomes (2, 3, 4, 8 and Z). The QTL region on the Z chromosome was large and including QTL for AFW, EW, EN and ESS. The significant QTL were located on chromosomes 2, 4, 8 and Z for WFE at position of 322, 156, 61 and 102 cM; on the chromosomes 3 and Z for AFE at position of 189 and 128 cM, on the chromosomes 4 and Z sex chromosome for EW at position of 191 and 76 cM; on the chromosomes 4 and Z for EN at position of 55 and 89 cM; on the chromosomes 2, 4 and 8 for HU at position of 89, 222, and 18 cM; on Z sex chromosome for ESS at position of 97 cM, respectively. A total of four significant QTL were detected at 5 % chromosome-wise significance level, while a total of 11 significant QTL were detected at 1 % genomic-wise significance level and the total variances explained by QTL were 10.7, 12.2, 18.6, 12.2, 15.3 and 5 % for WFE, AFE, EW, EN, HU and ESS, respectively. The additive effects attributable to QTL explaining 5.4 to 53.0 % for WFE, 1.6 % for AFE, 4.4 to 8.2 % for EN, 3.0 to 6.5 % for EW, -0.6 and -6.2 % for Hu and -55.6 % for ESS of the total phenotypic variance of the F_2 population, while the dominance effects attributable to QTL explained 0.3 to 10.5, 4.0, -1.1 to -18.0, -1.6, -3.9 to -5.3 for WFE, AFE, EN, EW and HU, respectively.

Keywords: Chickens, QTL, Microsatellite markers, Egg traits, Additive effects, Dominance effects

1. Introduction

The chicken genome consists of 38 pairs of autosomes and sex chromosomes Z and W and the chromosomes can be classified into two size groups, nine macrochromosomes and 30 microchromosomes [4]. The classic genetic map of chicken consisted of 119 loci of morphological mutations, biochemical polymorphisms or chromosome breakpoints, 44 of which had map positions [3]. The identification and utilization of QTL provide more rapid genetic improvement in selection programs, especially for traits that are difficult to improve with traditional selection programs [15]. Based on chicken linkage maps and data from a variety of populations, several studies have reported many QTL for egg traits in chickens [31, 32, 34, 35, 18, 28, 29, 25, 2]. However, [5] stated that the correlations of markers MCW0041, ADL0210, and MCW0110 with egg production traits were significant ($P<0.05$).

Recent development of statistical methods and comprehensive linkage maps of the chicken genome has provided tools for mapping loci affecting quantitative traits [21]. However, only few genome-wide QTL scans have been reported in poultry, and none of these has involved egg production and egg quality traits in layers. A better understanding of chicken QTL may facilitate the accurate selection of immature chickens. Therefore, MAS of immature females and males should greatly enhance genetic progress for egg character and production traits through accurate selection and accelerate genetic improvement at a young age. [28] using F2 population originated from a cross between Leghorn males and Rhode Island Red females, reported that: (1) the chromosome 1 was separated into four linkage groups, chromosome 2 into three linkage groups and the chromosome 5 into two linkage groups, (2) the linkage groups encompassed 800 cM of the autosomes based on the mapping function, (3) thirteen markers were mapped into a linkage group on the Z chromosome, encompassing 120 cM of the Z chromosome, (4) the total linkage map spanned 920 cM, and (5) the remaining 13 markers could not be assigned to a linkage group and were therefore excluded from the QTL analysis. In terms of egg production and eggshell quality, associations have been found for polymorphisms in the putative candidate genes of IGF-1, GH, and GHR in the growth hormone endocrine pathway [20, 24].

The resource populations used in the present study were generated by crossing males of Golden

Montazah (M) with females of White Leghorn (L). The main objectives were: (1) to phenotyping egg production and egg quality traits at different ages in the parental and F2 generations in such crossbreeding program, (2) to localize QTL affecting these egg traits in the F2 population using specific microsatellite markers, (3) to detect the chromosome group, number of informative microsatellite markers and chromosome map length (cM), and (4) to quantify the additive and dominance effects attributable to QTL.

2. Materials and methods

2.1 Breeding plan and experimental populations

A total number of 18 and 8 cockerels from Golden Montazah (M) and 64 and 51 pullets from White Leghorn (L) were chosen randomly and were used in crossing M males with L females. Each cock was mated with 10 hens and housed separately in breeding pen to produce F_1 crossbred chicks (½M½L), then inter-se matings were practiced to produce F_2 chicks with the genetic structure of $(½M½L)^2$. Also, purebreds from the two strains were produced. The breeding plan permitted to produce four genetic groups as presented in Table (1). Pedigreed eggs from each individual breeding pen were collected from the four mating groups. On the hatching day, chicks of all genetic groups were wing banded, brooded on the floor and were grown in open houses up to 16 weeks of age.

Table (1) Number of sires, dams and chicks for genetic groups used in the experimental work

Generation	Sire group	Dam group	Genetic group[+]	No. of sires	No. of dams	No. of hens
Parental	L	L	L × L	18	64	1002
Parental	M	M	M × M	8	51	775
F_1	M	L	(½M½L)	18	103	1343
F_2	F_1 or ½M½L	F_1 or ½M½L	$(½M½L)^2$	18	106	1011
			Total	62	324	4131

[+] L= White Leghorn, M = Golden Montazah; the sire denoted first in the genetic group.

All the chicks were vaccinated against common diseases and they were subject to the same managerial, hygienic and climatic conditions. During the growing and rearing periods, all the chicks were fed *ad libitum* a diet containing 23% crude protein and 3200 kcal ME /kg during the period from hatching to 6 weeks and a diet containing 23% crude protein and 2900 kcal ME /kg during 6 to 16 weeks of age. The detailed breeding plan and management of the experimental populations are presented by [17, 19] **.** Data of egg traits were age at first egg (AFE), weight at first egg (WFE), 120-days of egg number (EN), egg weight (EW), yolk weight (YW), albumen weight (AW), shell weight (SW), Haugh unit, and egg shell strength (ESS). Three consecutive eggs per month were collected for each hen from all genetic groups during 120-days of egg production.

2.2 Statistical analysis of the phenotypic data

The phenotypic data set of AFE, WFE, EN, EW, AW, YW, SW, HU and ESS were firstly analyzed using SAS program [27] to estimate the starting values of additive and residual variances to be used as prior values in the animal model analysis. The differences between means of the genetic groups were tested ($P<0.05$) and then, the data set was analyzed using multi-traits animal model of VCE6 program [10]**.** The animal model used in matrix notation was as follows:

$$y = Xb + Zaua + Zpup + e \quad \textbf{(Model 1)}$$

Where: y= n×1 vector of observation of the hen, n = number of records; X= design matrix of order n×p, which is related to the fixed effects of genetic group (four levels), year of birth (three levels) and hatch (two levels); b= p×1 vector of the fixed effects of genetic group, year and hatch; Za= the incidence matrix relating records to the additive genetic effect of the hen; ua= the vector of random additive genetic of the hen; Zp= the incidence matrix relating records to random permanent environmental effect of the hen; up= the vector of random permanent environmental effect of the hen; and e= n×1 vector of random residual effects, NID (0, $\sigma^2 e$). The heritability (h^2_a) was estimated using the following equation:

$$h^2_a = \frac{\sigma^2_a}{\sigma^2_a + \sigma^2 p + \sigma^2_e}$$ Where: σ_a^2, σ_p^2 and σ_e^2 are the variances due to the effects of direct additive genetic effect, permanent environmental effect and random error, respectively. The genetic correlations (rg) among the traits were estimated according to the formula: $$r = \frac{Cov\,(X)_{ij}}{\sqrt{[Var(X]_{ii}).Var(X_{jj})}}$$ Where: $Cov\,(X)_{ij}$ = the covariance between additive genetic effects for egg traits; X_{ii} and X_{jj} = the additive genetic (a) variances of ith and jth egg traits.

2.3 Blood sampling and DNA isolation for molecular genotyping

Blood samples (10 ml) were collected from the wing vein at 24 weeks of age from relevant mating birds of F_0 parents, F_1 and F_2 to be included in the genotyping panel. Blood samples were collected in vacuum tubes containing EDTA and stored at -20 ˚C until DNA extraction. Genomic DNA was extracted using the Maxwell® 16 blood DNA purification kit according to kit manual, designed specifically for the optimal automated extraction of DNA from whole blood samples on the Maxwell® 16 SEV Instrument. The quality and concentration of extracted DNA was examined spectrophotometrically.

2.3.1 Markers selected

A total of 45 microsatellite markers covering nine autosomal linkage groups and the sex Z chromosome were considered in genotyping fifty F0 grandparents, twenty F1 and two hundreds F2 offspring Table (2). These markers were selected based on the degree of polymorphism and the genome coverage recommended in the molecular genetic characterization of animal genetic resources [7]. Detailed information about the selected microsatellites are available at the FAO website (www.dad.fao.org/en/ refer/library/guidelin/marker.pdf). The assessment of markers was based on their positions on the consensus map. A target for marker spacing of 10 cM was used to test markers across the genome (http://www.ncbi.nlm.nih.gov/mapview and http://www.thearkdb.org).

2.3.2 PCR amplification

The PCR amplification was performed on a 25-µl reaction mixture (ready to use Master Mix Promega) containing 100–200 ng DNA template, 15 pM of each primer, 200 lM each dNTP, 1 U Taq DNA polymerase, and an optimized quantity of $MgCl_2$. The reaction was carried out by initial denaturation at 94 ºC for 2 minute, and then denaturation at 94 ºC for 30 second, annealing at the temperature optimized for each primer pair for 30 second and extending at 72 ºC for 30 second for 35 cycles, followed by a final extension step at 72 ºC for 5 minute. The optimum annealing temperatures for the best amplification are presented in Table (2). Amplified products were electrophoresed at Metaphor gel [23]. The gel was run with puc19 DNA marker at 120 V for 2 h in 1X TBE and stained with Ethidium Bromide. The gel was visualized and documented under a white light gel documentation system.

2.4 Statistical analysis of the molecular data (Linkage and QTL mapping)

A linkage map was generated using Map Manager QTX version b20 software program [22]. After parentage checking, data of 1011 chicks from F_2 individuals were genotyped using 45 microsatellite markers in nine autosomal linkage groups and Z chromosome and these genotypes were available for QTL analysis. Markers that did not meet the criteria of polymorphism were cancelled from the analysis. The linkage map analysis was used to get the best order of the markers, and to detect the map distance among the markers. The maps were then used for QTL detection on the autosomes, linkage groups, and the Z chromosome. Data of F_2 was used for analyzing the additive (a) and dominance effects (d) of QTL at a given position for each trait where the additive effect was defined as half the difference between the two homozygotes and the dominance effect as the difference between the means of the heterozygotes and homozygotes. Data of F_2 cross was analyzed using the following mixed model including the fixed effects of hatch along with the additive and dominance effects of QTL as random effects [11, 22].

$$\mathbf{y_{ij} = X_{ij}b + Z_a a + Z_d d + e_i \text{ (Model 2)}}$$

Where: y_{ij} is the phenotype of F_2 hens, X_{ij} is the designed matrix, and b is the vector of coefficients for hatch as a fixed effect, *a* is the vector of additive effect of the QTL, *d* is the vector of dominance effect of the QTL, Z_a the probability of one homozygous type at the putative QTL locus given the marker information minus the probability of the other homozygous type at the locus given the marker information for the hen i, Z_d is the probability of being heterozygous at the putative QTL locus given marker genotypes for the hen i, and e_i is the random error, typically assumed to be normally distributed as $N(0, \sigma^2)$. Detection of QTL was based on the value of F-statistic that was computed from sums of squares explained by the additive and dominance coefficients for the QTL. Additive and dominance effects were estimated for each putative QTL. The informativeness of the markers was assessed at each location. Significance thresholds at 1% and 5% levels, and confidence intervals were determined by Map Manager QTX software. Significant and suggestive QTL were defined by test statistics exceeding the 5% significance thresholds. The 5% chromosome-wise level threshold was used as suggestive QTL namely, $P_{genome} = \alpha/n$, where $\alpha = 0.05$, n was the total number of tests (traits x chromosome)

Table (2) Microsatellite markers used in genotyping birds of F_0, F_1 and F_2

Microsatellite marker (Locus)	Forward primer sequence	Reverse primer sequence	SSR (bp)*	T_m**
ADL0022	GCATCAGAGGAAGAAGGAAA	GCATCAGAGGAAGAAGGAAA	165	51
ADL0114	GGCTCATAACTACCTTTTTT	GCTCTACATTCCTTCAGTCA	185	45
ADL0142	CAGCCAATAGGGATAAAAGC	CTGTAGATGCCAAGGAGTGC	231	52
ADL0143	CCTGTCTCTGGTCTTTATCC	AGTTTACTTCCTTTTCTTGC	170	51
ADL0155	GGTCCGACTGAAAGCATTAT	TTAAGACTGAAGCCAACCAG	107	49
ADL0201	GCTGAGGATTCAGATAAGAC	AATGGCTGACGTTTCACAGC	143	53
ADL0217	TCTACTTCGTTGGAGTGTCA	GGAAAACAGAGGAGAAATGG	161	52
ADL0237	GCTTGTGCCTAAGAATGAAC	TGTATGGAGTCTCAGCAAAT	148	50
ADL0241	AAAATAGCATGGCAAATCAT	CAGATGCATCAGCACAGAAA	216	51
ADL0255	GGGTATTGGTCTTCAAAATG	GTAAAGGCCTTCCTCTTCTT	110	47
ADL0266	GTGGCATTCAGGCAGAGCAG	AATGCATTGCAGGATGTATG	113	50
ADL0322	TGCGTTCTCCCCTTGGTTGC	GCAGCAGCTCCCACGACACA	140	55
LEI0065	TGAAACATGTATGGAGTCTCAGCA	GACAGCTAAATGCCAGTTCATGG	187	61
LEI0072	TAAGCTGACATTCACCACCAG	GACTCTTTCAGTACATACTGG	100	63
LEI0073	TTGAGAGCAGTGAAGGCAAACG	TGGTGGGAACTGGAAGAAGAGG	217	65
LEI0075	TTTCACATCCAGTGCGTGTCTG	GGGCAGAGAAAGACGAAATTGG	188	65
LEI0081	ACTTACCTTTTCTTAGCTACTG	GATCCTTTCAATGCTCATGCT	260	61
LEI0111	CCCACAAAAGAGACACCGTGG	CCTGTTTGCCGTACACTTGGC	116	65
LEI0163	ACTTGGGCATACTCTTGTTGC	CTGCAGGTACCGTGAGATGTG	207	64
LEI0214	TGCCTCGTCTTACTGAGTGA	GATCAAGCACTGTATTTTATTC	164	60
LEI0254	AGACCACTGGATCCAACTC	GTCTGGAACTCATCCCTTCATC	95	55
MCW0004	GGATTACAGCACCTGAAGCCACTA	AAACCAGCCATGGGTGCAGATTGG	199	54
MCW0010	CTGTAGAATTACAGAAATACA	TAGTACAAGAATCTAGTGTTAAAA	93	45
MCW0045	CCAAAGGAAACAAATACTATACGA	GAAAGAAAAACTGACACTGTGACT	151	53
MCW0047	GGATTACGGCCGTTTGTGCACAAA	AATGGAACGCCGAACTCGCGTGCA	107	49
MCW0055	TTTGTAGTTACCTGGTACTGA	GTTTGCATTGTCTACAGCTCCTTG	193	51
MCW0056	TGGTAACCTCTAACCTTGACG	AGTGAAGGAGACTCCACAGCCTCT	207	48
MCW0083	TACATTTCAGAAGGAATGTTGC	GCCTTTCACCCATCTTACTGT	90	54
MCW0100	GATCTAAACAAAAACAGACACA	TGTAGGCGATTAAACATACTTC	90	55
MCW0107	GAACAGAACTCTGTTTACTG	TCTGCTTACCTCAACTGACA	121	56
MCW0122	TCCTTTGGAGCACGGAGGAAC	AGATGCACAGGCAGAGCTCCA	270	56
MCW0129	ATTTGGTGAACACAAACCTGC	CCACTTGAATGAAGCACCTAC	118	52
MCW0135	ATATGCTGCAGAGGGCAGTA	CATGTTCTGCATTATTGCTCC	150	57
MCW0154	GATCTGTTTTATCACACACAC	CCATTTCCTTTGTTATCAGGC	193	54
MCW0156	TCTGTAACATTTTTCCTTTTGTG	TTAATGTGGCAGACTCAAAGG	287	50
MCW0169	GATCCCACTTGTTAAGAAGTG	CCTGACCTTACTGAGCTTGGA	96	58
MCW0170	TTGTGAAACTCACAGCAGCTG	TTATAGCAGGCTGGCCTGAAG	177	52
MCW0180	GATCACATCACGTTAATTTT	GGTGGAGAAAAGTGAAAGAC	88	55
MCW0241	AACCAGTTTGTTAACATCAGC	ATTGGAGTTGGTACCATACTC	276	51
MCW0246	TCATAAGGCAGAGAATTCATC	TTTCCATTCAGACAACAAGGC	235	53
MCW0247	CTTCACATGCTCCACTTGATG	AGTGACTATACTTCTTCACGG	207	50
ROS0003	GCAAAGTTATTCAGGAACTTGC	AAGTGGTCCCCTGATTTAACA	250	56
ROS0026	GGCAAACACACAGTTTTCACA	ATGATCTCATGGAGTGCTGAGC	108	55
ROS0074	AGCACTTTTGGTGTTACCGG	CAGCTGATGCTTCCACAGAA	320	58
ROS0075	CAGCTCCGTGCTCCTCTC	TTTTCAACCCGTTGTTCAGG	216	58

* SSR = Simple sequence repeats; ** $T_{m=}$ Annealing temperature

3. Results and discussion

3.1 Phenotypic means of genetic groups

Hens of L had the superiority of for AFE and EN than M, while M had the superiority in WFE Table (3). The crossbreds were superior in egg production traits relative to the purebreds. The first cross of ½M½L had the superiority of egg production traits then the intercross of $(½M½L)^2$. As stated by [16, 6], egg traits of the present study indicated that local chickens in Egypt could be improved by crossbreeding.

Table (3) Means and standard errors (SE) for hen and egg traits in Golden Montazah (M), White Leghorn (L) and their crosses of chickens

Egg trait	Genetic group			
	M	L	½M½L	$(½M½L)^2$
	Mean ±S.E	Mean ±S.E	Mean ±S.E	Mean ±S.E
Age at first egg, AFE (days)	168.9±0.52^{a}	162.09±0.40^{bc}	158.31±0.49^{d}	161.07±0.49^{c}
Weight at first egg, WFE (g)	1566.3±20.9^{b}	1465.2±16.23^{c}	1825.4±19.7^{a}	1567.0±19.8^{b}
Egg number, EN (egg)	61.67±0.57^{d}	74.01±0.44^{c}	83.44±0.54^{a}	79.27±0.54^{b}
Egg weight, EW (g)	44.04±0.14^{a}	45.67±0.10^{c}	47.70±0.14^{b}	49.44±0.19^{a}
albumen weight, AW (g)	24.19±0.10^{d}	25.62±0.07^{bc}	27.22±0.09^{b}	28.10±0.13^{a}
Yolk weight, YW (g)	14.38±0.06^{c}	14.51±0.04^{c}	14.66±0.06^{b}	15.35±0.08^{a}
Shell weight, SW (g)	5.45±0.02^{a}	5.53±0.01^{b}	5.82±0.02^{b}	5.97±0.03^{c}
Haugh unit, HU	94.10±0.80^{a}	90.19±0.59^{b}	88.87±0.78^{b}	78.60±1.05^{ab}
Egg shell strength, ESS (%)	78.10±0.004^{a}	76.19±0.003^{b}	76.41±0.004^{b}	76.78±0.006

$^{a-d}$ Means with the same letters within each row of the trait are non-significantly different (P≤0.05).

Eggs of L breed were better than eggs of M strain in most egg quality traits (Table 3). But, M strain was better in HU and ESS compared to L breed. This may be due to differences in genetic makeup of the two strains [6]. Eggs of the crossbred hens were superior in most traits, probably due to genetic and non-genetic additive effects of genes. The eggs of F_2 cross had the heaviest egg weight, albumen weight, yolk weight and shell weight compared to F_1 cross.

3.2 Heritability and genetic (r_G) and phenotypic (r_p) correlations

Heritability estimates were 0.11, 0.11, 0.34, 0.14, 0.18 and 0.22 for AFE, WFE, EN, EW, ESS and HU traits, respectively and these estimates agreed with [16]. As AFE, WFE and EW are sex-limited traits and they are lowly to moderate heritable Table (4), they would greatly used as beneficial markers assisted selection, where the selection can be directed towards actual genetic variation.

The estimates of genetic correlation among the egg traits studied were mostly negative and low (8 estimates out of 15; Table 4). [31] reported that AFE had a phenotypic correlation with EW of 0.29 and it was -0.52 between AFE and EW.

Table (4) Heritabilities (diagonals), genetic (above diagonals), and phenotypic (below diagonals) correlations of investigated traits

Trait	AFE	WFE	EN	EW	ES	HU
AFE	0.11	0.01	-0.39	-0.04	0.12	-0.10
WFE	0.04	0.11	-0.28	0.13	0.00	-0.01
EN	-0.09	-0.26	0.34	0.15	-0.03	0.11
EW	0.01	-0.09	0.15	0.14	0.02	-0.94
ESS	-0.02	0.00	0.03	0.01	0.18	-0.06
HU	0.03	0.03	0.07	-0.84	-0.04	0.22

Traits as defined in Table (3).

3.3 Chromosomal linkage analysis

The total chromosomal map length was 1949 cM ranging from 52 cM on chromosome 11 to 542 cM on chromosome 1, with marker spacing ranging from 15.3 cM on chromosome 4 to 71.5 cM on chromosome 6 and average spacing of 43.3 cM Table (5). Map lengths for these chromosomes were considerably similar to those cited by [31] who stated that the linkage groups covered 2311 cM, and the estimates on the length of the complete genome ranged from 3064 to 3800 cM. and the QTL associated with egg numbers was linked to chromosome 8. [32] Found that number of QTLs were distributed over chromosomes, such as the QTLs for AFE on chromosomes 3 and Z for EW on chromosomes 2, 4 and Z**.** The QTL accounted for Hugh units were found on chromosome 1 [12], on chromosome 2 [31], and on chromosome 8 and 9 [26]. For egg weight and egg production, [29] showed two QTL regions on chromosome 2 and nine QTL on chromosome 4. QTL affecting egg number and egg weight were found in chromosomes 1, 2, 5, 6, 7, 8, 14, and Z [1, 5].

Table (5) Chromosome (linkage) group, number of microsatellite markers, chromosomal map length (cM), marker spacing on the chromosome and the first marker on each chromosome that was used for a whole genome scan of F_2 cross

Chromosome	Number of microsatellite markers used	Chromosome map length (cM)	Marker spacing on the chromosome (cM)	First marker on each chromosome
1	9	542	60.2	MCW0107
2	8	401	50.1	LEI0163
3	6	144	24	MCW0169
4	4	286	15.3	ADL0143
6	3	123	71.5	ADL0322
8	2	88	44	ROS0075
9	2	112	56	MCW0135
11	3	52	17.3	ROS0003
13	2	69	34.5	ADL0255
Z	6	132	22	ROS0074
Total	45	1949	Average = 43.3	

3.4 QTL mapping and confidence intervals

The position of QTL relative to the first marker indicated that QTL were located in the region of 61 to 322 cM, 128 to 189 cM, 76 to191 cM, 55 to 168 cM, 18 to 222 cM and 97 cM, for WFE, AFE, EW, EN, HU and ESS, respectively Table (6). In general, QTL mapping of the present study for egg production and egg quality traits are in agreement with some studies that have identified numerous QTL affecting these traits [31, 32, 13, 35, 9, 8]**.** Differences cited between different studies might be attributable to differences in: 1) crosses used in the various studies; 2) the ages of measurement of the traits among the studies and 3) the individuals would be of different physiological status caused at least in part by genetic differences.

The F-ratios for each QTL at chromosome-wise level showed that 15 out of 34 QTL were significant and these QTL were distributed over four distinct regions on five chromosomes (chromosomes 2, 3, 4, 8 and Z) as shown in Table 6 ($P < 0.05$ or $P < 0.01$), i.e. 15 significant QTL that affecting egg production and egg quality traits were located on five macro-chromosomes. For WFE, four significant QTL were located on chromosomes 2, 4, 8 and Z at position of 322, 156, 61 and 102 cM, respectively with 95% confidence intervals of 244-422, 144-185, 0-75 and 60-127 cM, respectively (Table 6). In this respect, two significant QTL were located on the chromosomes 3 and Z for AFE at position of 189 and 128 cM, respectively with 155-200 and 65-135 cM of the 95% confidence interval. For EW, two significant QTL were located on the chromosomes 4 and Z at position of 191 and 76 cM, respectively with 95% confidence intervals of 185-198 and 35-96 cM, respectively. For EN, three significant QTL were located on the chromosomes 4 and Z (two QTL on chromosome 4 and one on Z chromosome) at position of 55, 168 and 89 cM, respectively with 95% confidence intervals at 30-178 and 15-95 cM, respectively. Moreover, HU has three significant QTL located on the chromosomes 2, 4 and 8 at positions of 89, 222, and 18 cM, respectively, with 75-131, 211-224 and 0-21 cM of 95% confidence intervals. For ESS, one significant QTL was located on Z chromosome at position of 97 cM, with 77-134 cM at 95% confidence interval. [31] Stated that the 90% confidence interval for AFE was 65 to 137 cM on the chromosome 3, while it was 160 to 204 cM for EN on the chromosome 4. [14] Reported that the 90% confidence interval for the QTL location was broadened from the previous 58 cM to 64 cM despite the denser marker map. [8] cited that a genome-wide highly significant QTL for egg weight ($P < 0.01$) was identified on the chromosome 4 at 154 cM and the most interesting result of multiple QTL region on chromosome 4 between 19.2 and 82.1 cM and at least two QTLs in this region at 37.6 and 76.4 cM affected egg weight and a QTL at 58 cM affected the number of eggs. QTLs for egg weight were repeatedly discovered in a region between 59.9 and 82.8 cM [31, 28]. [29] also reported a QTL for egg weight between 62.1 and 75.8 cM in a cross between Broiler and White Leghorn; the favourable allele for egg weight came from the broiler strain. In a cross between Red Junglefowl and White Leghorn, a QTL for egg weight was identified on the same chromosome between 51.6 and 67.1 cM [18]**,** while [9] reported that QTL for AFE was found in the region around 130 cM on chromosome 1.

Table (6) Flanking markers, position of QTL relative to the first marker (cM), F-ratios and significance of QTL confidence interval of 95% (cM) for egg production and egg quality traits in F_2 population of chickens

Trait / Chromosome	Flanking markers	Position of QTL relative to the first marker (cM)	F-ratio for each QTL at chromosomal wise level	Confidence interval at 95% (cM)
Weight at first egg (WFE)				
2	ADL0114 - MCW0056	322	11.6**	244-422
4	ADL0241 - MCW0180	156	38.9**	144-185
8	MCW0100 - ROS0075	61	11.1**	0-75
Z	LEI0111 – LEI0075	102	8.9*	60-127
Age at first egg (AFE)				
3	ADL0155 – MCW0004	189	7.55**	155-200
Z	ADL0201-MCW0241	128	21.9**	65-135
Egg number (EN)				
4	MCW0047 – ADL0266	55	7.5**	30-178
4	ADL0266 – MCW0170	168	7.4**	30-178
Z	MCW0241 – MCW0246	89	14.22*	15-95
Egg weight (EW)				
4	LEI0081-MCW0122	191	27.18**	185-198
Z	ADL0022 – MCW0154	76	20.11**	35-96
Haugh unit (HU)				
2	MCW0247 – ADL0217	89	10.33**	75-131
4	MCW0180 - MCW0129	222	6.48*	211-224
8	ADL0322 - MCW0095	18	5.99*	0-21
Egg shell strength (ESS)				
Z	MCW0154-LEI0254	97	13.33**	77-134

Total QTL detected = 15.

*significant linkage at $P \leq 0.05$ and ** significant linkage at $P \leq 0.01$.

3.5 QTL at chromosome-wise and genomic-wise levels

Across the traits studied, a total of four significant QTL were detected at a 5 % chromosome-wise significance level, while a total of 11 significant QTL were detected at 1 % genomic-wise significance level Table (7). Also, the total variances explained by QTL were 10.7, 12.2, 18.6, 12.2, 15.3 and 5 % in WFE, AFE, EW, EN, HU and ESS, respectively. The whole genome scan for detection and localization of QTL affecting egg quality traits were described by [31] who found 14 chromosomal areas affecting egg quality at 1% genome-wise significance level, while at 5% level only 6 suggestive QTL were found. [33] Reported that 23 QTL affecting eggshell strength were found in the genome scan and genome-wide significant QTL were found on chromosomes 2, 6 and 14, and additional chromosome-wise significant QTL seem to cluster on these chromosomes and on chromosome 3. [26] found that QTL for shell strength was linked to chromosome 8 and linkage group 26 and there were several QTL found for all the measured egg production traits and most of the QTL are located on chromosomes 4 and Z. [8] found that the phenotypic F_2 variance for egg weights in the early and late production periods explained by the QTL at 93 cM and 154 cM ranged from 4.9 to 7.1% and 12.3 to 16.1%, respectively and the QTL allele contributed to early age at first egg explained 6.5% of the phenotypic F_2 variance.

Table (7) Number of significant QTL at 5 and 1% chromosome-wise level and genome wise level for each trait in F_2 cross

Trait	Chromosome-wise level		Genome-wise level		Variance (%)[+]
	5%	1%	5%	1%	
WFE	1	-	-	3	10.7
AFE	-	-	-	2	12.2
EW	-	-	-	2	18.6
EN	1	-	-	2	12.2
HU	2	-	-	1	15.3
ES	-	-	-	1	5
Total	4	-	-	11	-

[+] The sum of the total variances explained by the QTL in each trait.

3.6 Additive and dominance effects for QTL

The estimates of additive and dominance effects attributable to QTL for egg production and quality traits are given in Table 8. The additive effects attributable to QTL affecting WFE was found on chromosome 2, 4, 8 and Z and they were 85, 830, 109 and 95 g, explained 5.4, 53.0, 7.0 and 6.1% of the total phenotypic variance of the F_2 population, respectively, while the dominance effects were 5, 164, 36 and 15 g, respectively. For AFE, the additive effects attributable to QTL were found on the chromosome 3 and Z, accounting 2.5 and 2.8 day, while the dominance effect was 6.5 day for the chromosome 3 and with no dominance effect for the chromosome Z Table (8). The additive effects of two QTL affecting the egg number were found on the chromosomes 4 and Z, accounted for -6.5, -3.5 and -4.3 egg and explaining -8.2, -4.4 and -5.4 % of the phenotypic variance, respectively, while the dominance effects accounted -0.9 and -14.3 eggs for the chromosome 4 and with no dominance effect for the chromosome Z. [8] reported that the additive effects attributable to QTL were found on chromosome 7 at 154 cM and accounting from 1.9 to 2.4 g for egg weights and the dominance effects accounting 1.5 to 1.9 g at 93 cM, while the QTL additive effects on number of eggs were located on chromosome 7 and with QTL dominance effects on chromosomes 4 and 5.

The additive effects attributable to QTL were detected on the chromosomes 4 and Z and accounting 3.2 and 1.5 g and explaining 6.5 and 3.0 % of the phenotypic variance for egg weight; at the end of the chromosome Z and accounting −0.15 and explained 55 % of the total phenotypic variance for ESS; at chromosomes 2, 4 and 8 and accounting −4.9, -1.9 and -0.5 unit and explained -6.2, -2.4 and -0.6 % of the total phenotypic variance for Hu (Table 8). The dominance effects attributable to QTL were detected on chromosome 4 and accounting -0.8 g with no dominance effect for chromosome Z; with no dominance effect for ESS; on the chromosomes 2, 4 and 8 and accounting -3.5, -3.1 and 4.2 HU, respectively. [31] Found that Rhode Island Red (RIR) allele has an additive effect of −5.3 and -8.6 units for HU that explained 7 and 5% of the total phenotypic variance of the F_2 population, respectively. [14] Reported that the effect of the RIR allele was –3.73 for HU), while the dominance effect was –1.74 HU and the detected QTL explained 6.7% of the phenotypic variance.

4. Conclusions

(1) Significant QTL detected on chromosomes 2, 3, 4, 8 and Z concluded that there are different sets of genes affecting egg production and egg quality traits.

(2) Genome wide QTL mapping in F_2 populations clarified that the foundation for identifying the DNA variants are responsible for the variations in egg production traits in chickens, i.e. these QTL could be used for identifying the causative functional genes or to be used in marker assisted selection (MAS) in poultry improvement program.

(3) A single-QTL model could be used to detect different QTL locations in the same chromosome or on several chromosomes and further analysis using multi-trait QTL model might confirm these approaches of QTL.

Table (8) Estimates of additive and dominance effects (g) attributable to QTL and their standard errors (SE) for egg production and egg quality traits in F_2 population of chickens

Trait / Chromosome	Additive effect			Dominance effect		
	Estimate, unit	SE	VP_a (%) $^{+}$	Estimate, unit	SE	VP_d (%) $^{++}$
WFE (overall mean = 1567.0 ± 19.8 g)						
2	85	17.6	5.4	5	2.8	0.3
4	830	44.8	53.0	164	69.5	10.5
8	109	22.9	7.0	36	46.9	2.3
Z	95	30.5	6.1	15	8.5	1
AFE (overall mean =161.07 ± 0.49 g)						
3	2.5	1.1	1.6	6.5	2.2	4.0
Z	2.77	0.6	1.7	-	-	-
EN (overall mean = 79.27 ± 0.54 egg)						
4	-6.5	1.9	-8.2	-0.9	2.3	-1.1
4	-3.5	2.2	-4.4	-14.3	4.5	-18.0
Z	-4.3	1.3	-5.4	-	-	-
EW (overall mean = 49.44 ± 0.19 g)						
4	3.2	0.5	6.5	-0.8	0.6	1.6
Z	1.5	0.3	3.0	-	-	-
HU (overall mean = 78.6 ± 1.05 unit)						
2	-4.9	1.8	-6.2	-3.5	3.3	-4.5
4	-1.9	0.6	-2.4	-3.1	1	-3.9
8	-0.5	0.6	-0.6	-4.2	1.1	-5.3
ESS (overall mean = 0.27 ± 0.01 mm)						
Z	-0.15	0.04	-55.6	-	-	-

$^{+}VP_a$ (%) = Percentage of additive variance explained by each QTL.
$^{++}VP_d$ (%) = Percentage of dominance variance explained by each QTL.

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