IJABR Vol. 8(1): 151 - 159 (2017)

International Journal of Applied Biological Research 2017

Original Article

ANALYZING QUANTITATIVE TRAIT LOCI AFFECTING EGG QUALITY TRAITS ON CHROMOSOME 1-5 OF SHIKABROWN (*Gallus gallus domesticus,* Linnaeus 1758) F_2 CROSS

[1]Galadima, M. A., [2]Akpa, G. N., [3]Adeyinka, A. I., [3]Nwagu, B. I., [2]Kabir. M. and [1]Shehu, D. M.

[1]Department of Biological Sciences, Ahmadu Bello University, Zaria, Nigeria.
[2]Department of Animal Science, Ahmadu Bello University, Zaria, Nigeria.
[3]National Animal Production Research Institute, Ahmadu Bello University, Zaria, Nigeria.

Submitted: February, 2017; Accepted: April, 2017; Published: June, 2017

ABSTRACT

This study looked for genes affecting some egg quality traits on chromosome 1-5 and microsatellite markers attached to them to formed what is called quantitative trait loci, which can be use in marker assisted selection. Chromosome 1-5 of a population of 205 Shikabrown intercrossed (F_2) were scanned for Quantitative trait loci (QTLs) affecting egg quality traits, using 25 microsatellite markers. QTLs affecting shell thickness, yolk length, egg weight, yolk weight, albumen weight, shell weight, and albumen length were identified on these chromosomes. The effects of genotype on these traits were additive, while others were dominant. Two QTLs located on chromosome 2 (shell thickness and shell weight), and three QTLs located on chromosome 3 (albumen weight, yolk weight and egg weight) showed pleiotropic effect. Correlation between these QTLs were positive and significant. It was concluded that chromosome 1 to 5 of Shikabrown contain QTLs affecting egg quality traits.

Keywords: Shikabrown, Microsatellite, Chromosome, Additive Effect, Dominant Effect, Pleiotropy

***Corresponding author**: mogawal@yahoo.com, jid.hagala@gmail.com

INTRODUCTION

Shikabrown, bred at National Animal Production Research Institute Shika Ahmadu Bello University Zaria, hitherto is the only indigenous commercial breed of chicken. This breed of chicken was bred with the intention of obtaining good performance across the different ecological zones of this country. It served as good research material in that its productivity will be enhance through research and acquisition of knowledge on its biology.

Most economically important traits are under the influence of polygenes. Identifying those loci would underscore the kind of progress that would ensue in improving production of poultry and livestock at large. The knowledge on position and effect of quantitative trait loci (QTL) is lacking for most traits of interest to animal breeders. Identification and use of quantitative trait loci (QTL) in selection programmes offer the potentials for more rapid improvement, particularly traits that are difficult to measure. In order to enhance these potentials, identification of loci having large effects on these economically important traits and the origin of potentially beneficial alleles is necessary (Hocking *et al.*, 2002). Derived information on QTL is useful for marker assisted breeding, thereby improving the understanding of the biological background (that is which genes are involved and their effect) of traits (Vankaam *et al.*, 1999).

One of the major goals of agricultural research is the identification of genes controlling the expression of economically important traits. Most of these traits display a wide variation in the expression of genes at distinct loci (QTL). The currently availability of highly polymorphic DNA markers in many species renders, possible the elaboration of well saturated genetic maps and consequently, genetic dissection of complex quantitative trait (Wardecka *et al.*, 2003).

Conventional selection methods, using half sibs and pedigree information to estimate heritability and selection response is expensive, time consuming and cumbersome. Selection at molecular level is straightforward, needs fewer number of mapping population, less expensive and takes a shorter time to be accomplished. Information from these molecular markers linked to QTL of interest can be used in Marker Assisted Selection. Such procedure will bring faster gain in livestock improvement within the shortest time.

MATERIALS AND METHODS

The mapping population consisted of 205 F_2 generation obtained from crossing two chicken breeds. The sire line contained 252 hens and 25 cocks (Rhode Island Red), and the dam line contained 250 hens and 25 cocks (Rhode Island White), in the ratio of 1:10. The cocks of the sire line were crossed with the hens of the dam line using the same ratio. The chicks hatched were Shikabrown (F_1) that were identified, with pullets appearing brownish in color and the cockerels were white or silvery (auto-sexing). The F_1 Shikabrown chickens were crossed in the same ratio of 1:10 to produce the F_2 mapping population.

The birds were mated at the age of 40 weeks and the egg traits were monitored at the age of 45 weeks. Seven egg quality traits were recorded during the laying period. The traits were as follows: albumen weight, shell thickness, albumen length, egg weight, shell weight, yolk length and yolk weight.

Thirty-three microsatellite primers table (1) were used for the analysis

PCR conditions

The primers used in this study are presented in Tables 2 and 3. The PCR was carried out in a total of 20µL, containing 50ng genomic DNA , PCR buffer (10mM Tris-HCl, pH 8.8; 1.5 mM $MgCl_2$; 50mM KCL; and 0.1% Triton-X), 200 mM dNTP, 0.25 U Dynazyme II DNA polymerase and 10 pmol of each primer. The PCR was performed for 5 minutes. at 95^{o}C and subsequently with 25 cycles consisting of

the following steps: 30 sec at 95°C, 45 sec at an annealing temperature that varied from 43°C to 64°C depending on the marker, and 60 sec at 72°C, followed by a final elongation step of 5 minute at 72° C. Amplified products were multi-loaded on a 6 or 5.5% gel with ALF[7]or ALF Express[7]respectively. A mixture of 1μL of PCR product, 1 μL of internal standard, 4 μL of loaded dye (formamide) were denatured at 95°C for 2 min. Fragments analyses were performed with Fragment Manager Version 1.2. Software (Tuiskula-Haavisto *et al.*, 2002)

Sample Collection

Egg quality traits (yolk length, yolk weight, albumen weight, albumen length, Shell weight, shell thickness, and egg weight) table were measured. A total of 15 eggs per hen were collected. For each hen one egg was collected every seven days. Quality traits were measured on each egg and an average computed for each hen. The measurement were done using vernier caliper for (Yolk length and Albumen length), micrometer screw gauge for (Shell thickness) and sensitive electronic scale (KERRO BL20001, 0.05 gram sensitivity) along with petri dishes for (Egg weight, Yolk weight, and Shell weight). The unit used in measuring Yolk length Albumen length and Shell thickness is millimeter, while Yolk weight, Albumen weight, Egg weight and Shell weight were measured in grams.

Data Analysis

Linkage analysis

Linkage analysis was performed according to Tuiskula-Haavistor *et al.* (2002) using the CRIP-MAP program version 2 (Green *et al.,* 1990). All pair wise combination of markers was first analyzed by the TWOPOINT option. An LOD scored was assigned to indicate linkage and was used for configuration of a linkage group. Option FLIPS was used to check optional orders between adjacent markers. CHROMPIC was used to detect double or triple recombination and potential genotyping errors. Significance threshold was also determined. The chromosome-wide -P-value for suggestive linkage of a specific chromosome equals the contribution (r) of that chromosome to the total genome length, which was obtain by dividing the length of the chromosome by the total analyzed length of the genome (Tuiskula-Haavisto *et al.,* 2002)

Quantitative trait loci analysis

The mapping of QTL was performed using the program QTL cartographer version 1.13 (Basten *et al.*, 1999). The program uses linear regression, composite interval mapping (Zeng, 1993, 1994) method to dissect the underlying genetics of the quantitative traits. Composite interval mapping combines interval mapping with multiple regression (Wardecka *et al.*, 2003)

Significance thresholds were calculated using the permutation test (Churchill and Doerge, 1994). This is done by random shuffling of corresponding markers genotypes, for each trait. Any relationship between a marker and QTL is broken and reshuffled. The distribution under the null hypothesis of no QTL is constructed in this way.

Table 1: List of Primers Used in QTL Studies

1)MCW0132	12) MCW0115	23) MCW0115
2)MCW0247	13) MCW0252	24) MCW0040
3)MCW0082	14) MCW0037	25) MCW0139
4)MCW0095	15) MCW0001	26) MCW0107
5)MCW0003	16) MCW0063	27) MCW0114
6)MCW0018	17) MCW0131	28)MCW0047
7)MCW0120	18) MCW0220	29) MCW0081
8)MCW0068	19) MCW0206	30) MCW0217
9)MCW0128	20) MCW0041	31) MCW0029
10)MCW0145	21) MCW0127	32) MCW0032
11)MCW0252	22) MCW0126	33) MCW0193

RESULTS

Egg quality traits analysis for F_2 RHIRXRHIW crosses (Table 2), albumen weight had mean of 31.03± 0.01 with a coefficient of variation of 16.85%, the variation within this trait is low. The variation in Shell thickness 20.83%, albumen length 25.25%, yolk length 21.24% and yolk weight 20.71% were moderate. While egg weight 34.17% and shell weight 34.17% were high,

Table 2: Egg Quality Traits of F2 RHIR X RHIW Chickens

Trait	N	Mean±SE	CV %
Albumen weight	205	31.03±0.01	16.85
Shell weight	205	0.24±0.003	20.83
Albumen length	205	9.25±0.16	25.52
Egg weight	205	56.21±0.39	10.03
Shell weight	205	5.60±0.13	34.17
Yolk length	205	14.73±0.21	21.24
Yolk weight	205	15.45±0.22	20.71

Alb Wt=Albumen Weight, Shell Thc=Shell thickness, Alb Lth=Albumen Length,Egg Wt= Egg weight, Shell Wt= Shell Weight, Yolk Lth= Yolk Length, Yolk Wt=Yolk Weight.

Table (3) contains a linkage analysis of 25 microsatellite markers. Distances between loci were in kosambi scale within the mapped populations. The 25 microsatellites markers mapped on chromosome 1 to 5, gives a ratio of 5 haplotypes per chromosome. The polymorphism information content (PIC) of markers were on average 0.586. The linkage groups covered 913 centimorgan (cM), with an average spacing of 36.52cM between markers.

Phenotypic correlation was computed to evaluate the relationship between the egg quality traits (Table 4), Egg Wt was highly correlated to Yolk Wt (0.85), Alb Wt (0.93) and Alb Lth (0.82). There is a negative correlation between Yolk Length

and Shell Thc (-0.006), the correlation between Yolk Wt and Shell Thc was also negative (-0.089). The correlation between the rest of the traits ranged from moderate to low.

Table 3: Linkage Analysis of 25 Microsatellite Markers of F2 RHIR X RHIW Chicken

Position	Chromosome 1	Chromosome 2	Chromosome 3	Chromosome 4	Chromosome 5
Locus 1	MCW0018	MCW0063	MCW0127	MCW0107	MCW0081
(cM)	0.00	0.00	0.00	0.00	0.00
Locus 2	MCW0120	MCW0131	MCW0126	MCW0170	MCW0217
(cM)	100.00	28.61	43.99	100.66	21.93
Locus 3	MCW0068	MCW0220	MCW0115	MCW0114	MCW0029
(cM)	141.16	95.16	92.10	182.66	38.44
Locus 4	MCW0120	MCW0206	MCW0040	MCW0047	MCW0032
(cM)	172.13	138.98	134.42	241.36	57.11
Locus 5	MCW0145	MCW0041	MCW0139	MCW0298	MCW0193
(cM)	266.24	188.36	222.22	283.50	68.61

Table 4 : Correlations between Egg Quality Traits Perform on F2 RHIR X RHIW Chicken

Traits	Alb Wt	Shell Thc	Alb Lth	Egg Wt	Shell Wt	Yolk Wt
Alb.wt						
Shell thc	0.30					
Alb lth	0.53**	-0.21				
Egg wt	0.94***	0.30	0.82***			
Shell wt	0.51**	0.84***	0.55**	0.52**		
Yolk lth	0.47*	-0.06	0.69***	0.46*	0.22	
Yolk wt	0.79***	-0.09	0.53**	0.85***	0.17	0.55**

*=P<0.05, **=P<0.01, ***=P<0.001

Alb wt=Albumen Weight, Shell thc=Shell thickness, Alb lth=Albumen Length,Egg wt= Egg weight, Shell wt= Shell Weight, Yolk lth= Yolk Length, Yolk wt=Yolk Weight.

The threshold p-values for chromosome–wise QTL analysis of egg quality traits in the F_2 RHIR X RHIW (Table 5), show that all the seven traits were significant at 1, 5 and 10% levels of significance. However, only the 1% and 5% significant levels showed evidence of linkage between traits and markers.

The positions of QTLs affecting egg quality traits in cM on chromosome 1-5, are shown in Table 6. In total 10 chromosomes-wise QTL areas were identified. On chromosome 1, three QTLs

affecting shell thickness, yolk length and shell weight were identified. Shell thickness was linked to marker MCW145, yolk length was linked to marker MCW68 and shell weight linked with marker MCW145. A QTL affecting Albumen weight was discovered on chromosome 2 linked with marker MCW206 at about 138.9 cM.

Table 5: Threshold P-values for Chromosome-wise QTL Analysis of Egg Quality Traits in RHIR X RHIW F2 Chicken.

Trait	Threshold P-value		
	0.1	0.05	0.01
Egg weight (g)	8.67	18.98	35.01
Albumen wt. (g)	9.68	15.75	24.31
Albumen lth (mm)	10.55	17.04	25.94
Yolk weight (g)	10.45	14.98	26.47
Yolk length (mm)	8.78	17.04	25.94
Shell weight (g)	11.21	15.37	24.31
Shell thc. (mm)	10.37	19.77	23.40

Albumen wt=Albumen Weight, Shell thc.=Shell thickness, Albumen lth.=Albumen Length, Egg weight= Egg weight, Shell weight= Shell Weight, Yolk length= Yolk Length, Yolk weight=Yolk Weight

Table 6: Positions of QTLs (in cM) on Chromosome 1-5 and Their Corresponding, F-ratios, P-values and R^2 of Markers Attached to The QTLs

Trait	Chrom	QTL Position	Marker	Marker Position	P-value	R^2	Level of sig. %
Shell thic.		265.03	MCW 145	266.24	0.037	0.07	*
Yolk. lth.	1	140.96	MCW 068	141.16	0.020	0.21	*
Shell. wt.		265.07	MCW 145	266.24	0.001	0.09	**
Alb. wt.	2	136.99	MCW 206	138.94	0.016	0.23	*
Alb. wt.		219.71	MCW 139	222.22	0.031	0.26	*
Yolk. wt.	3	219.54	MCW 139	222.22	0.05	0.21	*
Egg. wt.		218.93	MCW 139	222.22	0.001	0.16	**
Alb. lth.		97.63	MCW 170	100.66	0.003	0.18	**
Alb.wt.	4	240.00	MCW 047	241.30	0.002	0.20	**
Shell.thc.	5	0.00	MCW081	0.00	0.008	0.09	**

* = $P < 0.05$, ** = $P < 0.01$

Alb wt=Albumen weight, Shell thc. =Shell thickness, Alb. lth.=Albumen length, Egg wt= Egg weight, Shell wt.= Shell Weight, Yolk lth= Yolk length, Yolk wt=Yolk weight.

The additive and dominance effect of genotypes across significant QTLs or Traits along with their linked markers on chromosome 1-5 are presented in Table (7). The AA (Rhode Island Red) genotype had an additive effect of 0.57 and dominance effect of -0.25 on shell thickness in chromosome 1, while the effect on yolk length had additive negative value -2.5 and a dominant positive value of 3.42. The effect of the AA genotype on shell weight is additive

negative -1.96 and the dominance effect is positive 2.77. In chromosome 2 the effect of AA genotype on Albumen weight and egg weight were positive with values of 2.21 and 3.51 while the dominance effect were both negative -1.95 and -1.79 respectively. The AA genotype has an additive effect of 3.09 and negative dominance effect of-0.88 on albumen weight , a negative additive effect of -2.21 and a positive dominance effect of 3.21 on yolk weight and a positive additive effect of 2.84 with negative dominance effect of -1.59 on egg weight in chromosome 3. The effect of the AA genotype on albumen length in chromosome 4 is additive negative-0.69 and dominance positive 2.95. While the effect of the genotype on albumen weight on the same chromosome is additive positive 3.38 and dominance negative -1.99. The BB (Rhode Island White) genotype had a positive additive effect of 0.61and a negative dominance effect of -0.092 on shell weight in chromosome 5.

Table 7: QTLs, Linked Markers, Genotypes and Their Corresponding Significant Additive and Dominance Effect on Chromosome 1-5 of F2 RHIR X RHIW Chicken

QTLs	Chromosomes	Marker Id	Genotypes	Additive Effect	Dominance Effect
Shell Thc	1	MCW145	AA	0.57	-0.25
Yolk Lth	1	MCW68	AA	-2.25	3.42
Shell Wt	1	MCW145	AA	-1.96	2.77
Alb Wt	2	MCW206	AA	2.21	-1.95
Alb Wt	3	MCW139	AA	3.09	-0.88
Yolk Wt	3	MCW139	AA	-2.21	3.21
Egg Wt	3	MCW139	AA	2.84	-1.59
Alb Lth	4	MCW170	AA	-0.69	2.95
Alb Wt	4	MCW047	AA	3.38	-1.99
Shell Thc	5	MCW81	BB	0.61	-0.09

Alb.wt =Albumen Weight, Shell the.=Shell thickness, Alb. lth.=Albumen Length, Egg wt.= Egg weight, Shell wt.= Shell Weight, Yolk lth.= Yolk Length, Yolk wt.=Yolk Weight

DISCUSSION

Quantitative trait loci study

The orders of 25 microsatellite markers in the linkage analysis were in agreement with the chicken consensus linkage maps (Groenen *et al.*, 2000). Two chromosome-wise significant QTLs affecting shell thickness and shell weight were found at exactly the same position 265cM on chromosome 1. The two traits were highly and positively correlated 0.838. The additive effect of the BB genotype on shell thickness is positive and the dominance effect is negative. The additive effect of BB genotype on shell weight is negative while the dominant effect is positive. This suggested that the two QTLs are not additive. The two traits were attached to a single QTL; thus pleotropic effect cannot be rule out at this QTL Sasaki *et al.*. (2004) had reported a region

on chromosome 1 affecting both shell thickness and shell weight in which breed of chicken?

Another QTL affecting yolk length located at 140.9 cM on chromosome 1 was discovered. The effect of the AA genotype on this QTL is negative additive-2.25 and the dominance effect is positive 3.42. This is suggesting that on the average, a chicken of the heterozygote genotype AB will have 1.17mm longer yolk than a chicken with a homozygote genotype of either AA or BB. This QTL accounted for 21% of the phenotypic variation in the trait, which was in agreement with the findings of Hansen *et al.,* (2005).

On chromosome 2 a QTL situated at 136.9 cM affecting albumen weight was located. The effect of the AA genotype on this QTL is positive additive 2.21 and negative dominance -1.95. This should not be surprising, because some QTLs affecting the same traits were identified on chromosome 3 and 4 with positive additive effect and negative dominance effect, signifying that each of the three loci, located on the different chromosomes (chromosome 2, 3, and 4) have contributed additively in affecting albumen weight. The three loci accounted for 20-26% of the phenotypic variance, which is consistent with what was reported by Schreiweis *et al.* (2005) and Sasaki *et al.* (2004). Three traits, albumen weight, yolk weight and egg weight were significantly linked with marker MCW139 at the same locus (219 cM) on chromosome 3 and the traits were highly correlated. The effect of the AA genotype on albumen weight is positively additive 3.09, while the dominance effect was negative -0.88. The effect of the same genotype on yolk weight is negatively additive -2.21, and positively dominant 3.21. On egg weight the effect is additively positive 2.84, and negatively dominant -1.59 implying that these traits are not additive in effect, but are influenced by only one QTL which has pleotropic effect on these traits. The QTL on chromosome 5 affecting shell thickness, located at 0.00cM was influenced by the BB genotype that had a positive additive effect 0.61 and a negative dominant effect -0.09. The effect of this genotype on the trait is additive.

CONCLUSION

Chromosome 1-5 contain genes affecting egg quality traits. The effect of some of these genes are additive, some are dominant, while others are pleotropic. Further analysis using higher density markers may fine-tune positions of markers that are at linkage disequilibrium with these traits loci and gives stronger resolution of QTLs that would be used in marker assisted selection.

REFERENCE

Basten, C. J., Weir, B. S. and Zeng, Z. B. (.1999.) *QTL Cartography Version. 1.13,* Raleigh, NC27: 695-75661.

Churchill, G. A. and Doerge, R. W. (1994). Empirical threshold values for quantitative trait mapping. *Genetics,* 13 (8):963-971.

Green, P., Falls, K. and Crooks, S. (1990). *Documentation for CRIP-MAP, Version 2.4*. Retrieved from http://www.biobase.dk/Embnetut/ Crimap.

Hansen, C., Nengjun, Y., Zheng, Y. M., Xu, S., Gavora, J. and Cheng, H. H. (2005). Identification of quantitative trait loci for

production traits in Chickens. *Animal Biotechnology,* 6:67- 79.

Hocking, P. M., Ikeobi, C. O. N., Woollies, J. A. Morris, D. R., Law, A. and Windsor, D. W. (2002). Quantitative trait loci affecting fatness in the Chickens. *International Society for Animal Genetics*, 33: 428-434.

Schreiweis, M. A., Hester, P. Y., Settar, P. and Moody, D. E. (2005). Identification of Quantitative trait Loci Associated with Egg Quality, Egg Production and BodyWeight in an F2 Resource Population of Chickens. *Animal Genetics,* 37:106-112.

Tuiskula-Haavisto, M., Honkatukia, M., Vilkki, J., de Koning, D. J., Schulman, N. F. and Maki-tanila, A. (2002). Mapping Quantitative Trait Loci Affecting Quality and Production Trait in Egg Layers. *Poultry Science. Journal*, 81: 919-927.

Vankaam, J. B. C., Groenen, M. A. M., Bovenhuis, H., Veenendaal, A. and Van Arendonk, J. A. M. (1999). Whole Genome Scan in Chickens for QTL Affecting Growth and Feed Efficiency. *Poultry Science Journal,* 78:15-23.

Wardecka, B., Olszewski, R., Zieba, G. and Pierzchala, M. (2003). Preliminary Mapping of QTL Affecting Egg Quality on Chromosomes 1-5 in Chicken. *Czechs Journal of Animal Science,* 3: 97-105

Zeng, Z. B. (1993). Theoretical Basis for Separation of Multiple Linked Gene Effect in Mapping Quantitative Trait Loci. *Natural Science,* 90: 10972-10976.

Zeng, Z. B, (1994). Precision Mapping of Quantitative Trait Loci *Natural Science,* 136:1457-1468.