

The current study was conducted to identify the quantitative trait locus (QTL) for the body weight at age 1, 7, 14, 21 and 28 days and daily gain at age 0-1, 1-2, 2-3 and 3-4 weeks, slighter carcass weight and tonic immobility in Japanese quail. Two divergently lines of wild and white Japanese quail which maintained in the Animal Science Research Center of the Shahid Bahonar University of Kerman was used. Birds in two lines were mated in reciprocal cross for generating F1 generation. The F2 generation was generated by random mating of F1 birds. The half sib and F2 model analysis of QTL express was used for QTL mapping the measured traits. Total F2 individuals (422 birds), their parents (34 F1 birds) and pure lines (16 birds) were genotyped by 2 informative microsatellite markers on chromosomes 4. On this chromosome, with the half sib model, no significant QTL at the 5%, 1% chromosome wide was detected. But, with the F2 model, a significant QTL at the 5% chromosome wide was identified for body weight (BW) at age 21 and 28 days, carcass weight and daily gain traits during 1-2, 2-3 and 3-4 weeks in 54cM position. The all of identified QTLs explain less of 4% of the phenotypic variance, because the traits of growth are complex traits affected by many loci influencing appetite, feed uptake, nutrient allocation, metabolic rate, physical activity and so forth.

KEY WORDS body weight trait, daily gain trait, Japanese quail, QTL mapping, tonic immobility.

INTRODUCTION

Rearing of the domestic fowl in Iran and its dissemination throughout the country has an old history. Iran (Persia) was a great empire from the $5th$ century BC to approximately the $7th$ century AD and extended from India (Delhi) to the Black and the Mediterranean seas. At those times and later, in the middle ages, Persia was located at the crossroads of major ways for transporting goods, including the domestic fowl, from the East to the West, both by land and waterways. Numerous wars in the territory of Persia and adjacent countries during those periods could also facilitate the spreading of the chicken populations. Archaeological exca-

vations confirmed the presence of the domestic fowl in the territory of Iran at the ancient times [\(Mohammadabadi](#page-5-0) *et al*. [2010\)](#page-5-0). According to [West and Zhou \(1989\),](#page-6-0) the chicken bones were found here in three regions: two findings in Tepe Yahya (southeastern Iran) dated at 3.900-3.800 BC and 1.000 BC, respectively, and one in Takht-e-Suleiman (northwestern Iran) dated at 1.000 BC. Persian merchants maintained strong trade ties with the Mediterranean area and sailed upstream the Volga river, reaching Nizhniy Novgorod in Russia. It is known that Persian chickens from the Guilan Province took part in the origin of the Russian Orloff breed ([Mohammadabadi](#page-5-0) *et al*. 2010). Since 1981, many centers were organized for reproducing native and commercial poultry varieties. One of these commercial varities is Japanese quail. The Japonica quail belongs to the Phasianidae family. It is wildly reared for egg and meat production in several countries of Asia, Europe and America ([Minvielle,](#page-5-1) 2004). Until recently, in contrast to chicken (*Gallus gallus*), only a few linkage groups have been known for the Japanese quail, and few genes have been mapped [\(Tsudzuki, 2008](#page-6-1)). Despite the large variety of traits that have been studied in the Japanese quail.

Therefore, improvement of economic traits in Japanese quails has been desirable response by traditional breeding programs. For example, the growth rate is improved based on phenotype, but advantages of molecular genetics are important in rapidly and useful changes, especially in quantitative traits such as meat quality [\(Tsudzuki, 2008](#page-6-1)). QTL refers to chromosomal regions that controlling quantitative traits. It may include one or a group of genes with weak or strong linkage. Many quantitative traits are controlled by a relatively large number of genes, these genes known as genes with a major effect. So, the QTL is the gene with major effect [\(Falconer and Mackay, 1996](#page-5-2)).

In chickens, the first report for QTL mapping appeared in 1998 on the basis of microsatellite DNA markers ([Vallejo](#page-6-2) *et al*[. 1998;](#page-6-2) [Van kaam](#page-6-3) *et al*. 1998). Science then, about 200 QTLs has been discovered for economic traits of chickens [\(Abasht](#page-5-3) *et al*. 2006).

Contrastingly, in Japanese quail there are at present only a few reports concerning QTLs, although QTL mapping is possible with DNA markers in addition to the mapping of traits. [Beaumont](#page-5-4) *et al*. (2005) tried to identify QTLs for body weight and 11 kinds of fear fullness-related traits with AFLP markers developed by [Roussot](#page-5-5) *et al.* (2003). Furthermore, [Minvielle](#page-5-6) *et al.* (2005) carried out QTL analysis using microsatellite markers developed by [Kayang](#page-5-7) *et al.* [\(2004\).](#page-5-7) [Esmailizadeh](#page-5-8) *et al.* (2012) have recently detected significant QTL for body weight (weight at 3, 4, 5 and 6 weeks of age) on chromosome 1 and 3 in Japanese quail. The extend of the microsatellites and AFLP markers has made it possible to detect new genes. This increased the interest of the quail in biological research. Also, the genetic maps were assistant researches for QTL mapping with microsatellites and AFLP markers in F2 and back cross populations [\(Minvielle](#page-5-6) *et al.* 2005). Because of, the body weight and carcass traits are the main of economic traits in Japanese quail, detecting QTL for growth traits is very important. Although Japanese quail in Iran have studied by molecular techniques for different purpose [\(Sohrabi](#page-6-3) *et al*. [2012;](#page-6-3) Ori *et al*[. 2014;](#page-5-9) [Moradian](#page-5-10) *et al*. 2014; [Mohammadifar](#page-5-11) *et al*[. 2009](#page-5-11); [Moradian](#page-5-12) *et al*. 2015), but until now researchers have not identified QTL affecting body weight, growth rate and important behavior trait on chromosome 4 in Japanese quail.

Moreover low energy expenditure, in addition to increased energy intake, has been a major cause of future weight gain, and variations in energy expenditure may be one of the underlying sources of variation in body weight [\(Moazeni](#page-5-13) *et al*. 2016). Various determinants, including body composition, hormonal levels, activity of the sympathetic nervous system, and genetics are responsible for differences in metabolic rate among individuals ([Moazeni](#page-5-13) *et al*[. 2016\)](#page-5-13). Hence, the objective of the present work was to identify QTL affecting body weight, growth rate and important behavior trait on chromosome 4 in Japanese quail and compassion of half sib and F2 model for detecting QTL.

MATERIALS AND METHODS

Experimental design

A F2 population was developed using two distinct Japanese quail strains, wild (meat type) and white (layer type). Eight pairs of white (S) and wild (W) birds were crossed reciprocally and 34 F1 birds (9 males and 25 females) were produced. The F1 birds including 17 SW and 17 WS progeny were generated by reciprocal crosses. The F1 birds were intercrossed randomly generating 422 F2 offspring (246 males and 176 females) in five continuing hatches. The F2 progeny was raised for 5 weeks on a floor covered with wood shavings in an environmentally controlled room with continuous artificial lighting and at a temperature was decreased gradually from 37 to 25 ˚C. The progeny received water and food *ad libitum*.

Phenotyping

Hatching weight, body weights at age 1, 2, 3 and 4 weeks and carcass slaughter weight were recorded on individuals of the F2 generation. The growth rate (average daily gain in body weight between consecutive ages) was derived based on the body weights. Tonic immobility was measured as the length of time during which a chick remained immobile by keeping it on its back for 10 s.

Genotyping

The 2 microsatellite markers on chromosome 4, based on the recognized microsatellite markers [\(Kayang](#page-5-7) *et al*. 2004) and their polymorphism information, were selected. Genomic DNA was isolated from blood samples using salting out method [\(Helms, 1990](#page-5-14)). Polymerase chain reactions for each marker were carried out separately in a reaction volume of 25 µL included 120 ng of template DNA, 2.5 µL 1X PCR reaction buffer, 0.5 μ*M* of each primer, 2 µ*M* of each deoxy nucleotide triphosphate (dNTP), 0.2 U of Taq polymerase and residual volume is deionizer water. The PCR products were electrophoresed in 6% polyacrylamide gels. A sample of bands (alleles) was shown in Figure 1.

Figure 1 An example of the band scoring ("1" and "2" the homozygous genotype, "3" the heterozygous individuals and "4" is ladder)

Fragment sizes were analyzed with TOTAL LAB analysis software (http://www.totallab.com). All genotypes were checked twice. In all, parents (16 birds), F1 (34 birds) F2 (422 birds) were genotyped.

QTL analysis

Phenotypic data were analyzed by using SAS software ([SAS, 2000](#page-5-8)). Means; standard deviation and coefficient of variation of traits were calculated. The QTL express software under half-sib and F2 models at http://qtl.cap.ed.ac.uk was used for QTL analyses. Data subjected to a model with sex and hatch as fixed effects in the model. The percentage difference in the residual sums of squares between the full and reduced model was calculated as the phenotypic variance, which that QTL could explain. Significant thresholds for analyses were calculated using a permutation test ([Churchill and Doerge, 1994](#page-5-15)). A total of 1000 permutations were computed to determine the empirical distribution of the statistical test under the null hypothesis of no QTL associated with the part of genome under study. Three significance levels were used: suggestive, 5% and 1% chromosome-wide [\(Lander and Kruglyak, 1995](#page-5-16)).

RESULTS AND DISCUSSION

Phenotypic data

A summary of descriptive statistics of the studied traits (including number of observations, minimum and maximum values, means and standard deviations) for the F2 generation derived from a reciprocal intercross between the wild and white lines are presented in Table 1. The average daily gain ranged between 11.3 g (for ADG3-4) to 0.59 g (for ADG0-1). The effect of hatch was significant $(P<0.05)$) for all of the traits studied while the sex of the bird had significant (P<0.05) effect on W3 and W4 and the related derived traits such as ADG2-3, ADG3-4.

The females were generally heavier than male and so the females were faster than the males in growth (P<0.05).

Half-sib analysis

The QTL for BW at hatch, 1 to 4 weeks of age and average daily gain for different consecutive ages and CW using the half-sib analysis model are presented in Table 2. The suggestive QTL were found for BW at age 3, 4 weeks and average daily gain at age 0-1, 1-2 weeks closed to marker CUJ0026 and for CW close to marker CUJ0074. F ratio for these QTLs were 1.01, 0.95, 1.24, 1.10 and 1.12.

In Figure 2, detected QTLs using the half-sib analysis model for studied traits with interval mapping model have been showed in chromosome 4.

F2 analysis

Information on suggestive and significant QTLs is presented in Table 3. Two main positions for detected QTLs identified on chromosome 4. The first is located close to marker CUJ0026 (22 cM), which contains QTLs associated with BW at age 2, 3 and 4 weeks f and average daily gain at age 1-2, 2-3 and 3-4 weeks. F ratios for suggestive and significant QTLs were 1.28, 2.25, 2.35, 2.79, 2.14 and 3.07, respectively. The second is located close to marker CUJ0074 (54cM) contains A significant QTL for CW with F ratio=2.82. Most additive effects of identified QTLs in this research were negative (Table 3). Only QTLs for body weight at age 1week, slaughter carcass weight and average daily gain 0-1week illustrated positive additive effects. Moreover, depending on the trait, dominance effects of QTLs were positive or negative. Detected QTLs explained 0.59-3.85% of the total phenotypic variance.

The detected QTLs for studied traits with interval mapping model on chromosome 4 was shown in Figure 2. Illustrated thresholds of QTLs on chromosome are suggestive $(P<0.10)$ and significant $(P<0.05)$.

Table 1 Statistics of the various traits for the F2 generation derived from a reciprocal intercross between the wild and white lines in studied Japanese quail

1 Numbers following body weight (BW) indicating age in weeks.

CW: carcass weight and ADG: average daily gain and numbers following them indicating consecutive age.

SD: standard deviation and CV: coefficient of variation.

¹ Numbers following body weight (BW) indicating age in weeks.

² Proportion of total variance explained by the quantitative trait locus (QTL).

CW: carcass weight and ADG: average daily gain and numbers following them indicating consecutive age.

† Denotes suggestive linkage.

* (P<0.05) and ** (P<0.05).

In this study, QTL were detected for body weight, slaughter carcass weight and daily gain which are important traits in poultry breeding. This research adds new information from a chromosome-wide map for QTL in Japanese quail, and it is the first report on the detection of loci affecting important economic traits on chromosome 4 in Japanese quail. The length of chromosome 4 is 55cM with two detected microsatellite markers [\(Kayang](#page-5-7) *et al*. 2004).

Two models of analysis were used for the QTL detection: the half sib and the F2 model. The F2 analysis model identified more QTL compared with the half sib analysis model. The detected QTLs with the F2 analysis model, 6 out of 7 suggestive QTLs in the chromosome wide were significant, whereas for the QTL detected with the half sib model, all of 5 QTL identified in the chromosome wide were in suggestive level. In general, the F2 analysis model is claimed to be more powerful than half-sib analysis, but only if the alleles in founder lines are fixed ([Weller, 2001](#page-6-1)).

The founder lines of the present experimental population were divergent Japanese quail strains (meat type wild and layer type white strains). Therefore, it is likely that the QTL alleles for BW were fixed in the founder population and F2 model were perfect to half sib model. The positive additive effects by F2 model indicate that alleles increasing body weight came from the meat type line and negative additive effects came from the layer line. Although, to our knowledge, there is no report on QTL for body weight related traits on Japanese quail chromosome 4.

Figure 2 Interval mapping of QTL using an intercross between two Japanese quail strains on chromosome 4 The lower and upper horizontal lines represent 5 and 1% chromosome-wide significant levels of linkage, respectively

| BW ¹ | Closed marker | Position (cM) | F ratio | OTL effect | | | OTL variance |
|--|-------------------------|-------------------------|------------------|--------------------------------|----------------------------|--------------------------------|----------------------------|
| | | | | (Additive $effect \pm SE$) | (Dominance) effectpm SE | (Imprinting $effect \pm SE$ | $\frac{9}{6}$ ² |
| BW ₀ | CUJ0026 | 22 | 0.69 | -0.142 ± 0.11 | -0.04 ± 0.16 | 0.03 ± 0.15 | 0.60 |
| BW1 | CUJ0026 | 22 | 0.68 | 0.09 ± 0.77 | 0.88 ± 0.83 | -0.11 ± 1.17 | 0.92 |
| BW ₂ | CUJ0026 | 22 | 1.28^{\dagger} | -2.43 ± 1.46 | 2.67 ± 1.56 | -1.25 ± 2.22 | 1.07 |
| BW3 | CUJ0026 | 22 | $2.25*$ | -4.53 ± 2.25 | 5.19 ± 2.43 | -4.18 ± 3.43 | 2.56 |
| BW4 | CUJ0026 | 22 | 2.35^* | -4.88 ± 2.79 | 4.35 ± 3.00 | -8.10 ± 4.30 | 3.85 |
| CW | CUJ0074 | 54 | $2.89*$ | 0.70 ± 1.80 | 1.78 ± 2.41 | -8.41 ± 3.03 | 3.39 |
| $ADG0-1$ | CUJ0026 | 22 | 0.92 | 0.02 ± 0.10 | 0.13 ± 0.11 | -0.02 ± 0.15 | 0.59 |
| $ADG1-2$ | CUJ0026 | 22 | $2.79*$ | -0.37 ± 0.13 | 0.27 ± 0.14 | -0.10 ± 0.20 | 0.87 |
| $ADG2-3$ | CUJ0026 | 22 | $2.14*$ | -0.31 ± 0.16 | 0.27 ± 0.17 | -0.39 ± 0.25 | 1.36 |
| $ADG3-4$ | CUJ0026 | ٢٢ | $3.07*$ | -0.12 ± 0.18 | -0.40 ± 0.22 | -0.66 ± 0.24 | 1.35 |
| Tonic immobility $\frac{1}{2}$ Numbers following hady waight (DW) indicating aga in weaks | CUJ0074 | 54 | 0.72 | -0.133 ± 0.09 | -0.10 ± 0.19 | 0.07 ± 0.06 | 0.68 |

Table 3 Quantitative trait loci for studied traits using the F2 analysis model on chromosome 4 in Japanese quail

 Numbers following body weight (BW) indicating age in weeks. ² Proportion of total variance explained by the quantitative trait locus (QTL).

CW: carcass weight and ADG: average daily gain and numbers following them indicating consecutive age.

† Denotes suggestive linkage.

* (P<0.05) and ** (P<0.05).

However, there are some of QTL studies that detected QTL for bodyweight on chicken chromosome 4. [Zhou](#page-6-4) *et al*. [\(2006\)](#page-6-4) reported a QTL affecting body weight at age 2, 4 and 6 weeks f and growth rate at age 0-2, 2-4 and 4-6 weeks in chromosome 4 of chicken. In addition, [De Koning](#page-5-17) *et al*[. \(2004\)](#page-5-17), [Sewalem](#page-5-18) *et al*. (2002), [Van Kaam](#page-6-5) *et al*. [\(1999\)](#page-6-5) and [Van Kaam](#page-6-3) *et al*. (1998) confirmed a QTL on

chromoosome 4 affecting body weight and feed intake found in a commercial broiler line.

In this study, the interesting observation was that suggestive and significant detected QTLs in this intercross explained only 0.43–3.85% of the phenotypic variance for body weight, rate growth and carcass traits by using each of two models. Growth is a highly complex trait affected by

many loci influencing appetite, feed uptake, nutrient allocation, body composition, physical activity and etc. This means that each locus affecting growth explains only a small fraction of the genetic variance.

CONCLUSION

In summary, commercial breeding programs of Japanese quail have become more complex and challenging because so many objective need to be simultaneously considered to reduce production costs, maintain health and improve product quality. Breeding goals must include increased growth rate, increased % breast weight, maintenance of good development and growth of the skeletal system and overall fitness. The relationships of these traits are complex and some of the traits are very difficult to measure. Therefore, molecular MAS may be required to improve genetic selection programs. The current study is the first step toward the fine mapping QTL affecting BW and average daily gain on chromosome 4. Following, valuable candidate genes may be found by combining results of fine mapping and the Japanese quail genome sequence, and further function study of the genetic background of growth and carcass traits of Japanese quail.

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