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ASSOCIATION OF SPOT 14 GENE POLYMORPHISMS WITH FEED INTAKE AND BODY WEIGHT IN LOCAL CHICKEN

F. A. Mohammed[1,3], A. F. Alkhalisy[1] and F. M. Hussein[2]

[1]Department of Public Health, College of Veterinary Medicine, University of Baghdad, Iraq.
[2]Office of Agricultural Research, Ministry of Agriculture, Iraq.
[3]Department of Al-karkh Agriculture, Sector of Animal Wealth, Governorate of Baghdad, Iraq.

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ABSTRACT : Spot 14 gene plays important roles in chicken growth, it's a small acidic protein that responds to thyroid hormone stimulation and therefore its play a role in growth. The purpose of the present study was to analyze association of the Spot 14 gene with feed intake and body weight in local chicken. DNA from blood samples was extracted to amplify the MC4R gene and purified by polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP), and investigated its association with economic traits and statistical data were analyzed using SAS program. There was significantly affect (P<0.05) of the Spot 14 gene polymorphism on mean of the weekly live body weight and weekly feed intake in all weeks, the genotype BB recorder the highest mean followed by genotype AB and AA

Key words : Spot 14 gene polymorphism, chicken.

INTRODUCTION

The human diet plan in the modern scenario lays emphasis on consumption of protein rich diet as per who norms. Chicken is a major source of protein rich diet which continuous as high as 54% protein per 100 gm (Bhalla *et al*, 2015). Because of many years of domestication and breeding, a range of wide variety of chicken breeds exists today. However, because of many malpractices and constraints including limited availability of feed, lack of subsidies, religious sentiments, limited poultry feed ingredients, and poor knowledge regarding poultry farming, an expanding number of local breeds are under danger of eradication rendering numerous genotypes and traits at risk of being extinct (Blackburn, 2006).

The generation of a high quality draft sequence for the genome of chicken (Gallus gallus) is an important advance. Chickens are good models for studying the genetic basis of phenotypic traits, because of the extensive diversity among domestic chicken selected for different purpose. Monogenic traits are well-studied (Dodgson and Romanov, 2004; Nicholas, 2003), but many interesting traits are complex and determined by an unknown number of genes. However, in the modern poultry industry, introgression from non-commercial chickens is rarely used, resulting in very narrow genetic and allelic diversity in commercial versus non-commercial breeds. Since only a few breeds were used in formation of modern lines, local breeds have essential roles in maintenance of genetic diversity and to ensure future success and sustainability of the poultry sector (Muir *et al*, 2008; Bodzsαr *et al*, 2009).

Genetic mapping places molecular genetic markers in linkage groups based on their co-segregation in a population. The genetic map predicts the linear arrangement of markers on a chromosome and maps are prepared by analyzing population derived from crosses of genetically diverse parents, and estimating the recombination frequency between genetic loci (Duran *et al*, 2009).

The chicken Spot 14 was first identified by microarray analysis as a differentially expressed sequence tag in livers of chickens divergently selected for fast or slow growth rate (Cogburn *et al*, 2000; 2003a,b).Expression of Spot 14 mRNA is regulated by the thyroid hormone status in broiler chickens (Wang *et al*, 2002). Duplicated polymorphic paralogs of Spot 14, Spot 14α, Spot 14β were reported in the chicken (Wang *et al*,2004). Despite low similarity in amino acid sequence between chickens and mammals, other properties of Spot 14 (i.e. pl, subcellular localization, transcriptional control, and functional domains) appear to be highly conserved. Furthermore, a synteny group of Spot 14 and its flanking genes [reduced form of nicotinamide-adenine dinucleotide

dehydrogenase (NDUFC2) and glucosyltransferase (ALG8) appears to be conserved among chickens, humans, mice, and rats (Wang *et al*, 2004). Expression of thyroid hormone responsive Spot 14 alpha gene (Spot 14α) is up regulated by thyroid hormone, a prime regulator of body weight and growth (Campbell *et al*, 2003). Furthermore, the protein product of Spot 14α function as a transcription factor on lipogenic genes' promoters, thus maintaining an association between thyroid hormone concentrations and lipogenesis and growth (Hirwa *et al*, 2010).

In Iraq, chicken meat is considered highly preferable meat in many communities and due to lack of data regarding the genetic maps of local chicken, so this study was conducted to investigate the association of MC4R gene with feed intake and body weight in local chicken.

MATERIAL AND METHODS

Experimental animals:This study was conducted in the Poultry Research Station (20 km west of Baghdad) / State Board for Agricultural Researches/ Ministry of Agriculture. A total of 150 chickens were screened. Blood sample was drawn from wing vein and prepared for DNA isolation by using the procedure suggested by (Hoelzel, 1992). Each accession 2ml blood were taken as source of DNA a stored in K3 EDTA tubes and stored of -20 ºC.

The optimal condition has identified for (Initial denaturation and annealing) after a work several experiments to gain for this condition, the temperature has changed through the work of (Gradient PCR) for all samples to select the optimal condition, and also changed the concentration for DNA template between (1.5-2µl) where is considered these two factors from important factors in primer annealing with complement.

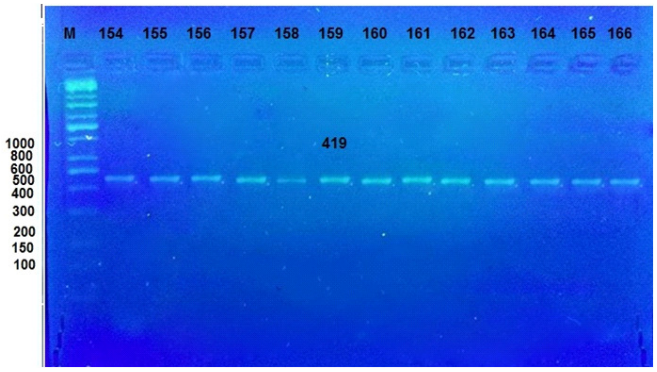

Figure 1 : PCR product the band 419 bp.

INtRON'sMaxime PCR Pre Mix Kit has not only various kinds of PreMix Kit according to experience purpose, but also a 2X Master mix solution. Maxime PCR Pre Mix Kit (i- Taq) is the product what is mixed every component: i-Taq DNA polymerase, Dntpmixture, reaction buffer, and so on-in one tube for one rxn PCR. This is the product that can get the best result with the most convenience system. The first reason is that it has very components for PCR, so we can do PCR just add a template DNA, primer set, and D.W.. The second reason is that it has Gel loading buffer to do electrophoresis, so we can do gel loading without any treatment. It is suitable for various sample's experience by fast and simple using method.

Statistical analysis: The Statistical Analysis System-SAS (2012) program was used to effect of Spot 14 and MC4R genes genotype in production and physiological parameters of Locale chickens. Least square means (General Linear Model procedure) and Duncan's (1955) Multiple Range test was used to significant compare between means, as well as extracting the distribution ratios of the herd and the frequency of the alleles obtained Chi-Square test of genes based on Hardy and Weinberg low; (Edwards, 2008). The statistical model was as follows:

$Y_{il} = \mu + G_i + e_{ij}$, Where, Y_{ij}: dependent variable ; µ: overall mean; G_i: Effect of genotype (AA-AB - BB of Spot 14 gene and GG- GC - CC of MC4R gene); and e_{ij}: Error term.

RESULTS AND DISCUSSION

Amplification of the target region of Spot14 and MC4R genes: The target region of Spot 14 gene (419 bp), were extracted by using PCR technique by PCR kit, primers, samples of DNA and turn PCR program, and by using DNA marker (100-2000 bp) in first pore of gel, and put the gel in 1 x TBE buffer. The product was electrophoresis on agarose gel 2% at 5 volt/cm². 1ãTBE buffer for 1:30 hours. N: DNA ladder 100.

The product was electrophoresis on 2% agarose at 5 volt/cm^2. 1x TBE bufferfor 1:30 hours. N: DNA ladder (100).

Distribution of genotype and allele frequency of Spot 14 gene in localchicken: The results in Table 2 refer to the distribution of different genotypes of spot 14 gene in

Table 1 : Specific primer of SPOT 14 gene.

Primer	Sequence	Tm (ºC)	GC (%)	Product size
Forward	5'-CAG GAG GGA GCA GAG GGA TAG - 3'	58.5	61.9	419bp
Reverse	5'- CGT CGT CCA AGA CTG GCT GG - 3'	60.5	65	

local chicken that showed high significant differences (P<0.01) in rate of three genotypes. The genotype AA showed a high percentage reached to 66.00% percentage follow by genotype BB as 22.00% percentage, while genotype AB showed a least percentage (12.00), and allele frequency of A was dominant on allele frequency of B that reach to 0.72 and 0.28 as A and B allele respectively. As the law of (Hardy Weinberg equilibrium). The Chi-Square analysis revealed that association of Taq1 allelic pattern with strain was significant.

Table 2 : Distribution of genotype and allele frequency of SPOT14 gene in local chicken

Genotype	No	Percentage (%)
AA (Wild)	66	66.00
AB (Heterozygous)	12	12.00
BB (Mutant)	22	22.00
Total	100	100%
Chi-Square (X^2)	—-	96.480 **
Allele	Frequency	
A	0.72	
B	0.28	
** (P<0.01).		

The results of (Table 3) showed significant differences among the three genotypes of Spot 14 gene in weekly body weight of local chicken in the third week, fourth week and fifth week by BB genotype that dominant on the other genotypes as 165.09 gm, 246.36 gm and 343.27gm, follow by AA genotype in third week as 157.56 gm and AB genotype in fourth and fifth weeks as 233.83 gm and 324.33 gm respectively, while there were non-significant differences with body weight in the weight at hatching, first week, second week, third week, sixth week, seventh week and eighth week.

The effect of Spot 14 gene on feed intake in (Table 4), there were significant differences (p<0.05) by BB genotype that reached 76.46 gm and followed by AB genotype as 74.24 gm, while the others weeks were non-

Table 3 : Relationship of the Genotype of SPOT 14 gene with weekly body weight.

Body weight:	Mean ± SE of body weight (gm)			Level of sig.
Weeks	AA (No. = 66)	AB (No. = 12)	BB (No. = 22)	
Wt. at hatching	34.63 ± 0.63	32.67 ± 1.51	35.41 ± 1.45	NS
Week 1	53.95 ± 0.82	53.83 ± 1.50	55.22 ± 1.60	NS
Week 2	95.68 ± 1.29	99.08 ± 4.08	99.68 ± 3.21	NS
Week 3	157.56 ± 2.09 b	158.91 ± 5.98 ab	165.09 ± 5.02 a	*
Week 4	236.68 ± 3.00 ab	233.83 ± 8.89 b	246.36 ± 5.19 a	*
Week 5	331.01 ± 4.11 ab	324.33 ± 12.39 b	343.27 ± 9.06 a	*
Week 6	440.44 ± 5.24	430.16 ± 15.70	453.36 ± 11.01	NS
Week 7	562.71 ± 6.33	550.91 ± 18.52	575.73 ± 13.26	NS
Week 8	698.34 ± 7.47	685.16 ± 21.65	711.23 ± 15.73	NS

* (P<0.05), NS: Non-SignificantMeans having with the different letters in same row differed significantly.

Table 4 : Relationship of the Genotype of SPOT 14 gene with weekly feed intake

Feed intake-	Mean ± SE of FI (gm/bird)			Level of sig.
FI: Weeks	AA (No. = 66)	AB(No. = 12)	BB (No. = 22)	
Week 1	39.06 ± 0.16	38.81 ± 0.37	39.49 ± 0.26	NS
Week 2	75.36 ± 0.42 ab	74.24 ± 0.91 b	76.46 ± 0.74 a	*
Week 3	125.54 ± 1.17	123.18 ± 2.49	128.59 ± 2.07	NS
Week 4	181.33 ± 0.83	179.73 ± 1.81	183.54 ± 1.44	NS
Week 5	230.61 ± 1.77	227.36 ± 3.93	235.31 ± 3.00	NS
Week 6	278.06 ± 3.35	271.67 ± 7.30	286.93 ± 5.77	NS
Week 7	326.76 ± 3.47	319.58 ± 7.23	335.61 ± 6.24	NS
Week 8	420.13 ± 4.72	410.67 ± 10.01	432.41 ± 8.32	NS
Total FI	1676.90 ± 15.86 b	1645.26 ± 33.84 b	1718.37 ±27.75 a	*

* (P<0.05), NS: Non-SignificantMeans having with the different letters in same row differed significantly.

significant differences by others genotypes.

The total feed intake showed significant differences ($p<0.05$) in BB genotype as 1718.37 gm that dominant on AA and AB genotypes as1676.90 gm and 1645.26 gm respectively.

Growth is under complex genetic control, and uncovering the molecular mechanisms of growth will contribute to more efficient selection for growth in chickens (Deeb and Lamont, 2002).Thyroid hormone belongs to the hypothalamic-pituitary-thyroid axis, and the hypothalamic-pituitary-thyroid axis plays an important role in the regulation of energy homeostasis via the effects of TH to increase oxygen consumption and heat generation (deJesus *et al*, 2001). Disturbance of thyroid function is associated with marked changes in body weight and energy expenditure (Belsing *et al*, 2003). The mammalian Spot 14 gene can respond to thyroid hormones quickly (Cogburn *et al*, 2003b). In chicken, the expression of THRSP m RNA increase dramatically in the liver of newly hatched chicks as they begin to synthesize and deposit abdominal fat (Cogburn *et al*, 2003). The result mentioned that the THRSP gene has an effect on lipid and lipoprotein processing at different stages of chicken development.

Referring to the in vivo and in vitro cell culture, respectively, to liver and abdominal fat, THRSP gene expression responds to both but more slightly to glucose than T3. Foufelle and Ferre (2002) reported the regulation of hepatic glucose metabolism as a key to the whole body energy metabolism because the liver is able to store and to produce glucose.

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