

## **THE POLYMORPHISM AMONG FOUR LOCAL CHICKENS IN LEI0258 MICROSATELLITE MARKER**

Questan Ali Ameen<sup>\*</sup>, Ali Hasan Mohamed<sup>\*\*</sup>, Sanaa Gazei Omar<sup>\*\*\*</sup>, Rana M. Al-Obaidi<sup>\*\*\*\*</sup>, Sehand  
K. Aref<sup>\*\*\*\*\*</sup>, Ahmed Sami Shaker<sup>\*\*\*\*\*</sup>, Hani Naser Hermiz<sup>\*\*\*\*\*</sup>

<sup>\*</sup> Animal production department, college of agriculture, Sulaimani University, Sulaimani, Iraq.

<sup>\*\*</sup> Department of veterinary microbiology, College of veterinary medicine, University of Kirkuk, Kirkuk, Iraq

<sup>\*\*\*</sup> Animal production department, Technical institute-Mosul, Northern technical university, Iraq

<sup>\*\*\*\*</sup> College of veterinary medicine, Sulaimani University, Sulaimani, Iraq.

<sup>\*\*\*\*\*</sup> College of Science, Biology Department, Sulaimani University, Sulaimani, Iraq.

<sup>\*\*\*\*\*</sup> Animal production department, Directorate of agricultural research, Sulaimani, Iraq.

<sup>\*\*\*\*\*</sup> Animal production department, college of agriculture, Salahaddin university, Erbil, Iraq

(Received 27 march 2021, Accepted 26 April 2021)

**Keywords:** Key words: Immune, MHC, chicken.

**Corresponding author;** [kosrat\\_ahmed@yahoo.com](mailto:kosrat_ahmed@yahoo.com)

### **ABSTRACT**

Major histocompatibility complex (MHC) is a cluster of genes found in vertebrate. In chicken it's located in chromosome 16, exactly linked with LEI0258 microsatellite marker (Accession no. KF534927.1). Current study was done to sequence the LEI0258 microsatellite marker in four genetic groups of Kurdish local chicken. Which was namely Black, Black brown neck, White shank feather, and White non-feathering shank. Fresh blood samples were withdrawn from wing vein and genomic DNA was isolated from each to determine the sequences. The result showed that the sequences of the four genetic groups differ from each other. Two clusters were found among the genetic groups the first content Black and black brown neck, and the second cluster content White shank feather and the white non-feathering shank.

## INTRODUCTION

Kurdish local chicken is an important source of meat and eggs in the rural population. It's adaptive to the regional climate and resistance to the diseases (1). These chicken genetic groups were faithfully characterized by several researchers according to their production traits, including egg traits (2, 3, 4, 5, 6, 7, 8, 9,10), carcass traits (11, 12, 13), and reproductive traits (14).

Major histocompatibility complex (MHC) is a cluster of genes found in vertebrates (15, 16, 17), moreover its multigene family. In chicken MHC gene was used as an indicator for blood group, also named B complex, and several investigators founded that it is important element for viral such as Marek (18, 19,20), Newcastle disease (21,22), and also for bacterial disease resistance (23,24).

MHC located in micro-chromosome 16 in chicken, and it's composed of two regions B and RFP-Y. B complex is mainly composed of three loci which different functionally BF, BL, and BG (25). A BG locus is unique for birds and its presence on the surface of red blood cells (26). Moreover, this locus is located on the (LEI0258) microsatellite marker which known to be physically located within the MHC first identified by (27). Therefore, it is necessary to assess genetic information of indigenous breeds, because it can provide an inexpensive alternative for MHC genotyping, and also can be used as a preliminary screening in any population as well as characterized populations in chicken breeds. The aim of our study is to sequence the LEI0258 microsatellite marker, which is linked with MHC traits in four genetic groups of the Kurdish local chicken in Kurdistan region-Iraq.

## MATERIALS AND METHODS

The current study was carried out in February 2019 in the animal science department laboratories, College of agricultural sciences at Sulaimani University, and the animal production department in the

directorate of agricultural research in Sulaimani, which associated with the ministry of Agriculture in KGR-Iraq. Blood samples were collected from chicken at the animal production department in the directorate of agricultural research in Sulaimani. A total of forty local chickens were representing Black (B=10), Black brown neck (BBN= 10), white shank feathering (WSF=10), and white non-feathering shank (WNFS=10). The genetic groups used were described faithfully by (4). At least 2.5 ml of a fresh blood sample was withdrawn from the wing vein from each chicken in an EDTA tube, (23) gauge needle. The blood was gently mixed with anticoagulant, and kept on ice. Subsequently, the blood samples were transported to the laboratory and stored at  $-20^{\circ}$  C until the isolation of genomic DNA.

Genomic DNA was isolated using a commercial kit, AccuPrep<sup>®</sup> Genomic DNA Extraction kit, with slight modifications. Then the DNA samples quality and quantity were evaluated by spectrophotometer (Nano-Drop2000, Delaware USA), based on 260 and 280 nm absorbance, and agarose gel electrophoresis analysis.

The PCR primers forward (5'-CACGCAGCAGAACTTGGTAAGG-3') and reverse (5' AGCTGTGCTCAGTCCTCAGTGC-3') were used to amplify the LEI0258 microsatellite marker. A total volume of 10  $\mu$ l was used, and the PCR reaction contained about 50 ng genomic DNA. The PCR program included an initial denaturation step at 94  $^{\circ}$ C for 5 min followed by 30 cycles of 94  $^{\circ}$ C for 30 sec, 55  $^{\circ}$ C for 30 sec., extension at 72  $^{\circ}$ C for 30 sec. and a final extension at 72  $^{\circ}$ C for 10 min (28).

## **RESULTS AND DISCUSSION**

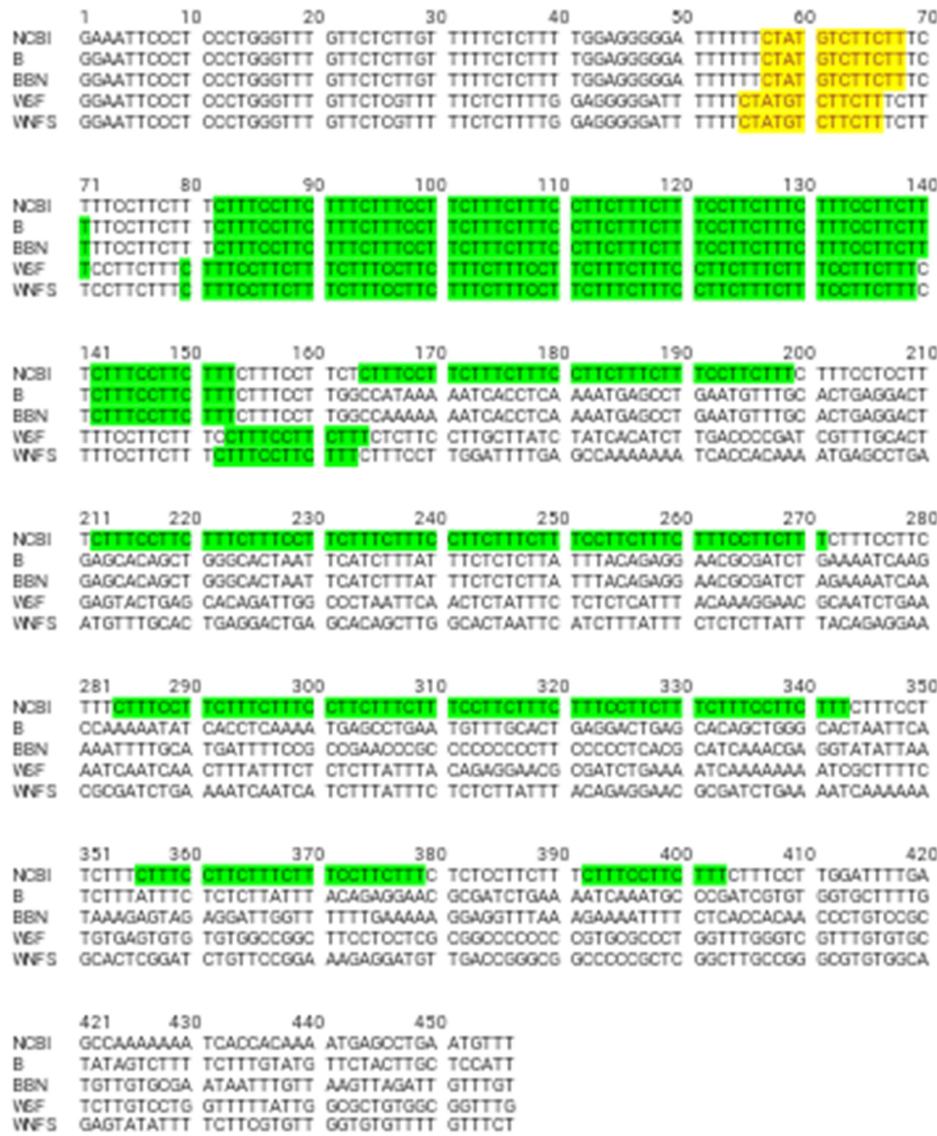
Figure (1) showed the sequence of LEI0258 in the four studied lines of the local Kurdish chickens compared with the sequence of LEI0258 in NCBI database. As studied by (27), the marker LEI0258 has two motifs R12 and R13 (R12: CTTTCCTTCTTT, R13: CTATGTCTTCTTT). Our study showed that the repeat region contained 2 repeat fragments, a 13-bp repeat of CTATGTCTTCTTT and a 12-bp

repeat of CTTTCCTTCTTT. The R13 appeared only 1 time, while 7 to 25 times for R12 table (1). Wang, (26) found that R13 repeated 1 to 28 times, and R12 repeated 2 to 20 times by using different chicken breeds. Moreover Han, (29) recorded by using indigenous chickens that R13 repeated 1 to 20 times, and R12 3 to 27 times.

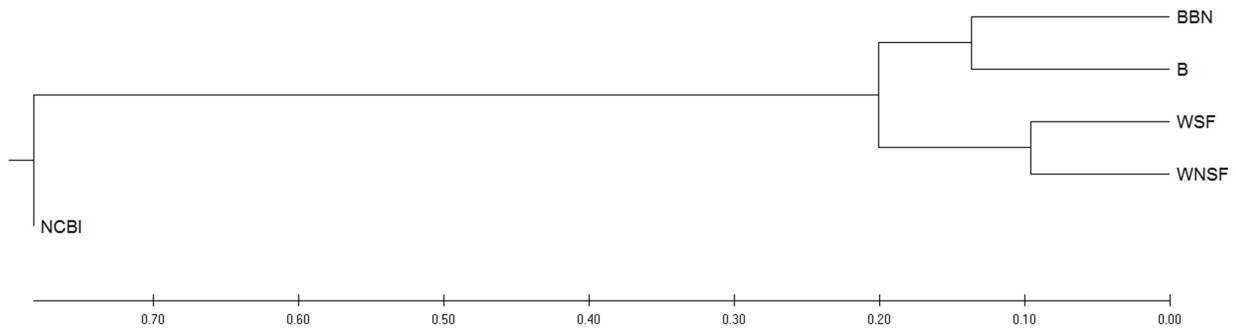
Depending on the sequence of LEI0258 in each genetic group and NCBI database sequence, phylogenetic analysis was done (Figure 2), which is shown three clusters. The first cluster for the Black and Black brown neck lines. The second cluster for the white feathering shank and white non-feathering shank. The third cluster was for the NCBI sequence (Ac: KF534927.1). These clusters mainly depend on the morphological characteristics of the four chicken lines as (30) find in his study by using same lines. Mpenda, (31) found that the cluster was formed depending on the geographic origin. Moreover, Izadi, (32) observed variation between the industrial chickens compared to non-industrial populations when studied the variability in the MHC alleles between the populations.

**Table (1): The repeats of R13 and R12 motives among the four lines of local Kurdish chickens**

Line	R13 CTATGTCTTCTTT	R12 CTTTCCTTCTTT
Black	1	7
Black brown neck	1	7
White shank feather	1	8
White non-feathering shank	1	8
NCBI (Ac. KF534927.1)	1	25



Figure(1: Informative SNPs found in the LEI0258 marker among the four genetic groups of the local Kurdish chicken and the LEI0258 sequence in NCBI (Ac. KF534927.1)



**Figure 2:** Phylogenetic analysis of LEI0258 among four genetic groups of local Kurdish chicken and NCBI sequence (Accession no. KF534927.1)

## CONCLUSION

In conclusion, the results of this research provided information on the genetic diversity of these chicken populations that can be used in decision making on conservation and in developing breeding stocks for free run/free range production.

## تعد الاشكال الوراثية بين اربعة سلالات للدجاج المحلي في الواسمة LEI0258

كويستان علي امين، علي حسن محمد، سناء غازي عمر، رنا محمد العبيدي، سهند كمال الدين عارف، احمد سامي شاكر،

هاني ناصر هرمز

### الخلاصه

معقد التوافق النسيجي الكبير هو مجموعة من الجينات الموجودة في الفقريات . في الدجاج يكون موقعة في الكروموسوم ١٦ و مرتبط تماما بالمعلمة الوراثية LEI0258 . أجريت الدراسة الحالية لمعرفة تسلسل القواعد النايتروجينية لأربع مجاميع وراثية من الدجاج المحلي الكوردي. وهي الاسود و الاسود ذو الرقبة البنية و الابيض ذو الساق المتريشة و الابيض ذو الساق عديمة الريش. تم سحب عينات الدم من وريد الجناح و عزل الحامض النووي لتحديد تسلسل القواعد النايتروجينية. حيث اظهرت النتيجة ان تسلسل المجموعات

الرابعة تختلف عن بعضها البعض. تم تحديد مجموعتين و احتوت كل منهما على سلالتين . المجموعة الاولى ضمت الاسود و الاسود  
ذو الرقبة البنية، اما المجموعة الثانية فضمت الابيض ذو الساق المتريشة و الابيض ذو الساق عديمة الريش.

## REFERENCES

1. **Ri-Fu, X., Kui, L., Guo-Hong, C., Yangzong, Q., Yu-Bo, Z., Li, L.** Genetic variation within Exon 2 of the MHC B-LB II gene in Tibetan chicken. *ActaGeneticasinica*, 35 (11), 2005, 1136-1146.
2. **Abas, K. A., Hermiz, H. N., Al-khatib, T. R., Amin, S. M., Ahmed, A. M., & Hamad, D. A.** Comparative productive performance of local hens in Erbil-Kurdistan region. *Journal of zankowsulaimani*, 6 (special issue), 2014, 203-206.
3. **Hermiz, H. N., Abas, K. A., Al-Khatib, T. R., Amin, S. M., Ahmed, A. M., Hamad, D. A.** Effect of strain and storage period on egg quality characteristics of local Iraqi laying hens. *Research opinions in animal and veterinary science*, 2 (1), 2012, 98-101.
4. **Shaker, A. S., Hermiz, H. N., Al-Khatib, T. R., & Mohammed, R. M.** Egg shape characterization for four genetic groups of Kurdish local chickens. *Food and nutrition science-an international journal*, 1, 2016, 20-25.
5. **Shaker, A. S., & Aziz, S. R.** Internal traits of eggs and their relationship to shank feathering in chicken using principal component analysis. *Poultry science journal*, 5 (1), 2017, 1-5.
6. **Aziz, S. R., Shaker, A. S., & Kirkuki, S. M.** Changes in external egg traits of chicken during pre- and post - molting periods. *Poultry science journal*, 5 (2), 2017, 9-13.
7. **Shaker, A. S., Kirkuki, S. M., Aziz, S. R., & Jalal, B. J.** Influence of genotype and hen age on the egg shape index. *International journal of biochemistry, biophysics, and molecular biology*, 2 (6), 2017, 68-70.
8. **Abdullah, S. M., & Shaker, A. S.** Principal component analysis of internal eggs traits four genetic groups of local chicken. *Egypt. Poult. Sci.*, 38 (2), 2018, 699-706.
9. **Hermiz, H. N., Shaker, A. S., Abas, K. A., Sardary, S. Y., Ameen, Q. A., & Al-Khatib, T. R.** Egg production evaluation for Kurdish local chicken in two different environments and estimates of their genetic parameters. *International journal of advances in science engineering and technology*. 7 (4), 2019, 72-75.



- 10. Shaker, A. S., Mustafa, N. A., Ameen, Q. A., Hermiz, H. N., Saadullah, M. A., Ramadan, A. A.** Egg traits uniformity comparison between Kurdish local chicken and two commercial strain using coefficient of variation. *International journal of advances in science engineering and technology*. 7 (4), 2019, 62-65.
- 11. Hermiz, H. N., Sardary, S. Y., Al-Khatib, T. R., Salih, S. J., & Shaker, A. S.** Comparison study of carcass traits in roosters resulted from different local lines and their crosses with ISA Brown. *International journal of advances in science engineering and technology*. 4 (3), 2016, 186-189.
- 12. Hermiz, H. N., Ameen, Q. A., Shaker, A. S., Sardary, S. Y., & Al-Khatib, T. R.** Principal component analysis applied to live body weight and carcass traits in ISA Brown and Kurdish local roosters. *International journal of advances in science engineering and technology*. 7 (1), 2019, 42-45.
- 13. Hermiz, H. N., Shaker, A. S., Ameen, Q. A., Sardary, S. Y., & Al-Khatib, T. R.** Predicting the carcass uniformity in Kurdish local chicken by using coefficient of variation. *International journal of advances in science engineering and technology*. 7 (1), 2019, 54-57.
- 14. Hermiz, H. N., Shaker, A. S., Hasafa, B. M., Al-Khatib, T. R., Sardary, S. Y., & Toma, J. S.** Evaluation semen characterization of roosters resulted from different local lines and their crosses with ISA Brown. *International journal of agricultural science*, 1, 2016, 7-14.
- 15. Babik, W., Durka, W., & Radwan, J.** Sequence diversity of the MHC DRB gene in the Eurasian beaver (castor fiber). *Molecular ecology*, 14, 2005, 4249-4257.
- 16. Hosomichi, K., Shiina, T., Suzuki, S., Tanaka, M., Shimizu, S., Iwamoto, S.** The major histocompatibility complex (MHC) class IIB region has greater genomic structural flexibility and diversity in quail than the chicken. *BMG Genomics*, 7, 2006, 322.
- 17. Lunney, J. K., Ho, C.-S., Wysocki, M., & Smith, D. M.** Molecular genetics of the swine major histocompatibility complex, the SLA complex. *Developmental and comparative immunology*, 33, 2009, 362-374.
- 18. Hansen, M. P., Van Zandt, J. N., & Law, G.** Differences in susceptibility to Marek disease in chicken carrying two different B locus blood group alleles. *Poultry Science*, 46, 1967, 1268.
- 19. Briles, W. E., Stone, H. A., & Cole, R. K.** Marek's disease: effect of B histocompatibility alloalleles in resistance and susceptible chicken lines. *Science*, 195 (4274), 1977, 193-195.



20. Bacon, L. D., Hunt, H. D., & Cheng, H. H. Genetic resistance to Marek's disease. *Current topics in microbiology and immunology*, 255, 2000, 121-141.
21. Lwelamira, J., Kifaro, G. C., Gwakisa, P. S., & Msoffe, P. L. Association of LEI0258 microsatellite alleles with antibody response against Newcastle disease virus vaccine and body weight in two Tanzania chicken ecotypes. *African journal of biotechnology*, 7 (6), 2008, 714-720.
22. HakoTouko, B. A., Keambou, T. C., Han, J. M., Bembide, C., Cho, C. Y., Skilton, R. A. The major histocompatibility complex B (MHC-B) and QTL microsatellite alleles favorable effect on antibody response against the Newcastle disease. *Int. J. Gen. Res*, 1 (1), 2013, 1-8.
23. Lamont, S. J., Bolin, C., & Cheville, N. Genetic resistance to fowl cholera is linked to the major histocompatibility complex. *Immunogenetics*, 25 (5), 1987, 284-289.
24. Cotter, P. F., Taylor, R. L., & Abplanalp, H. B-Complex associated immunity to salmonella enteritidis challenge in congenic chicken. *Poultry science*, 77 (12), 1998, 1846-1851.
25. Delany, M. E., Robinson, C. M., Goto, R. M., & Miller, M. M. Architecture and organization of chicken micro chromosome 16: order of the NOR, MHC-Y, and MHC-B subregions. *Hered*, 100, 2009, 507-514.
26. Wang, H., Ma, T., Chang, G., Wan, F., Liu, X., Liu, L. Molecular genotype identification of different chickens: Major Histocompatibility Complex. *The open access journal of science and technology*, 2, 2014, 1-7.
27. Fulton, J. E., Juul-Madsen, H. R., Ashwell, C. M., McCarron, A. M., Arthur, J. A., O'Sullivan, N. P. Molecular genotype identification of the Gallus galls major histocompatibility complex. *Immunogen*, 58, 2006, 407-421.
28. McConnell SK, Dawson DA, Wardle A, Burket T. The isolation and mapping of 19 tetra nucleotide microsatellites in the chicken. *Anim Genet* 30, 1999, 183-189.
29. Han, B., Lian, L., Qu, L., Zheng, J., & Yang, N. Abundant polymorphisms at the microsatellite locus LEI0258 in indigenous chickens. *Poultry science*, 92, 2013, 3113-3119.
30. Shaker, A. S., Ameen, Q. A., Al Obaidi, R. M., Arif, S. K., Sleman, S. S., Nore, B. F., Hermiz, H. N., Kirkuki, S. M. S., Al-Khatib, T. R. Estimation of Genetic Diversity in Four Genetic Groups of Local Kurdish Chicken Using RAPD-PCR. *Al-Anbar journal of veterinary sciences*, Vol. 13 (2), 2020, 101-108

- 31. Mpenda, F. N., Tiambo, C. K., Kyallo, M., Juma, J., Pelle, R., Lyantagaye, S. L.** Association of LEI0258 marker alleles and susceptibility to virulent Newcastle disease virus infection in Kuroiler, Sasso, and local Tanzanian chicken embryos. *Journal of pathogens*, 2020, 1-8.
- 32. Izadi, F., Ritland, C., & Cheng, K. M.** Genetic diversity of the major histocompatibility complex region in commercial and noncommercial chicken flocks using the LEI0258 microsatellite marker. *Poultry Science*, 90,2011, 2711-2717.