Plant Archives Volume 20 No. 2, 2020 pp. 6662-6664 **e-ISSN:2581-6063 (online), ISSN:0972-5210**

IDENTIFICATION OF LACTOFERRIN GENE POLYMORPHISM IN IRAQI NATIVE CATTLE USING PCR-RFLP TECHNIQUE

Zainab Sabeeh Al-Allak[1]*

Department of Animal Production, College of Agriculture University of Misan, Misan Province, Iraq.

Abstract

A total of 30 cows from three dairy herds in Misan province were used to obtained polymorphism of bovine lactoferrin (LTF) gene for a possible genetic marker information. Two alleles A, B were found in the examined population. The alleles controlled the occurrence of two genotypes AA, AB. Statistical analysis showed that there was no Hardy-Weinberg equilibrium between the observed and expected distribution of LTF genotype. It was found that polymorphism existed in bovine LTF gene, which suggested that it could be associated with somatic cell count (susceptibility/resistance mastitis).

Key words: Bovine lactoferrin gene, polymorphism, PCR-RFLP, Iraqi cows, mastitis.

Introduction

It is known that genetic merit for traits of animal health and reproduction is in opposition to merit for production traits. Thus, it is of great importance to discover associations between genes related with animal health and performance simultaneously. Lactoferrin (LF), an iron-chelating protein, is present in many mammalian biological fluids, including milk. Lactoferrin is a potential genetic marker for mastitis resistance, due to the following reasons: this protein plays a crucial role in immune response during mastitis (*e.g.* Rainard & Riollet 2005); the magnitude of its expression is connected to the udder health state (*e.g.* Chen & Mao 2004); and, importantly, the LF gene and its promoter are highly polymorphic. Since LF exhibits potential for further application as a mastitis resistance/susceptibility marker and selection for mastitis resistance cannot interfere with selection for dairy performance (which would diminish milk production profitability) there is a need to evaluate whether LF is associated with dairy performance traits. The unfavorable effect of LF on milk traits may possibly make it an inferior mastitis marker. Only a few papers have so far included bovine chromosome 22 (in which the LF gene is localized) among the regions of importance for milk traits (Ashwell *et al.*, 2004, Kolbehdari *et al.*, 2009). There are, however, reports that the LF genotype influences dairy performance, apart from its impact on SCC (Kamiñski *et al.*, 2006, O'Halloran *et al.*, 2010). Due to the above-mentioned facts, the aim of this paper was to investigate the genetic polymorphism of LF gene in local Iraqi cattle for the first time because of the close relationship between LF gene and resistant to mastitis and other dairy production trait as we mentioned above. Mastitis represents true threat in local Iraqi cattle and can be consider one of the main causes of reduction in milk production, beside infection causes, there must be a specific study identify the genetic causes of mastitis in which LF is the most important one.

***Author for correspondence :** E-mail : zainabsabeeh76@gmail.com

Materials and methods

Blood samples of 30 Local bovines were obtained from the jugular vein. Animals were in lactation period, samples collected in EDTA tubes (3ml) from each animal and kept in refrigerator for further investigation. Genomic DNA was extracted from the whole blood using the salting out method [Sambrook and Russel, 2002 The LTF genotypes were identified with the PCR-RFLP technique. The isolate DNA was used for PCR amplification of the LTF gene fragment of 301 bp with the use of the selected primer. Sequences of the primers that were used in PCR were reported previously by wojdak-Maksymiec *et al.*, [5]. Sequences of LTFR and LTFF were 5' CAGGTTGACACATCGGTTGAC 3' and 5' GCC TCATGACAACTCCCACAC 3', respectively. The PCR mixture contained 2µL of DNA, 2µL 10X PCR buffer, 10

pmoL of each primer, 1.5 mM Mgcl2, 200 µM dNTP, 1 unit Taq DNA polymerase and sterilized distilled water to make a final volume of 20 µL. Conditions for PCR were 94°C for 2 min, followed by 32 cycles of 94°C for 60 s, 61°C for 45 s and 72°C for 60s. The final step was at 72°C for 5 min. PCR products were digested with *Eco*RI enzyme (Fermentas Co.) which were used for determination of LTF A and B alleles. Eight microliters PCR products was digested with five units of (Eco R1) enzyme in 20 µL of reaction at 37°C for 6 h for RFLP of the LTF gene. Restriction fragments were analyzed electrophoretically in 2% agarose gel in TBE buffer for 2 h, and the genotype bands were visualized under UV light.

Results and discussion

In this experiment, PCR-RFLP method was utilized as a useful approach for LTF genotype marking and it could be considered as selection criterion in dairy cattle population. The primers used in this study were similar to those utilized by Wojdak-Maksymiec *et al.*, in Polish Black and White cattle (Wojdak-Maksymiec *et al.*, 2006). The AA, AB alleles of the LTF gene were identified based on amplification of specific primer (LFTR and LFTF), followed by digestion with the restriction enzyme *Eco*RI. Fig. 1, represent PCR product amplification of LTF gene after gel electrophoresis.

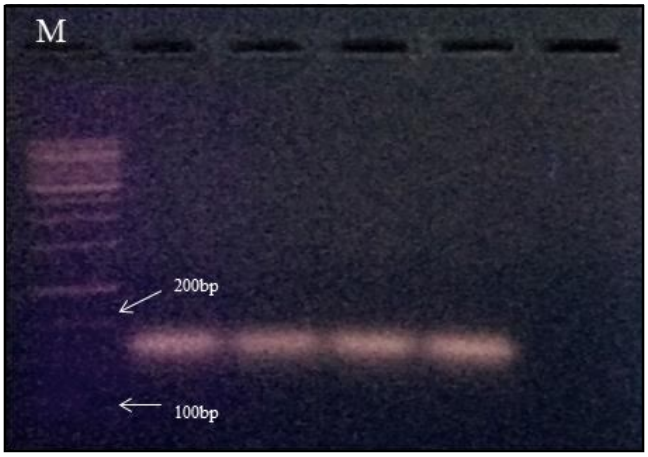

Fig. 1: Gel electrophoresis of PCR product. From left to right; lane 1 = molecular size markers; lanes 2-5 = PCR expression.

Two genotypes AA, AB of the LTF gene were observed in this experiment. Allele A was characterized by (125) bp fragment while the allele B was (175) bp. The frequency of the AA, AB genotype in the all herds was observed Fig. 2.

The result of *t* test indicated that statistically significant ($p<0.01$) deviation were found in the studied population between the observed and expected distribution of LTF genotypes, according to the Hardy-Weinberg equilibrium. Previously, it was stated that LTF gene can control the broad-spectrum antimicrobial activity, especially against coliform bacteria, such as *Escherichia coli*, which cause severe mastitis in dairy cows. Several studies have investigated regards association between bovine LTF gene polymorphism and SCC of milk (Wojdak-Maksymiec *et al.*, 2006; Sender *et al.*, 2006; Šrubaøová *et al.*, 2009) According to the Wojdak- Maksymiec *et al*., there is an association between bovine LTF gene polymorphism in the intron 6 region and SCC in Polish Black and white dairy cows, that reported animals with AA genotypes are associated with lower SCC than AB groups (Wojdak-Maksymiec *et al.*, 2006). However, Sender *et al*., provided contrary results and reported genotype BB animals showed the lowest SCC than the other groups (Sender *et al.*, 2006). Because of the low frequency of the BB genotype, investigation on this polymorphism need to be continued. Another investigation for polymorphism of bovine LTF gene in the same region has been reported by Šrubaøová and Dvoøák, who showed two genotypes

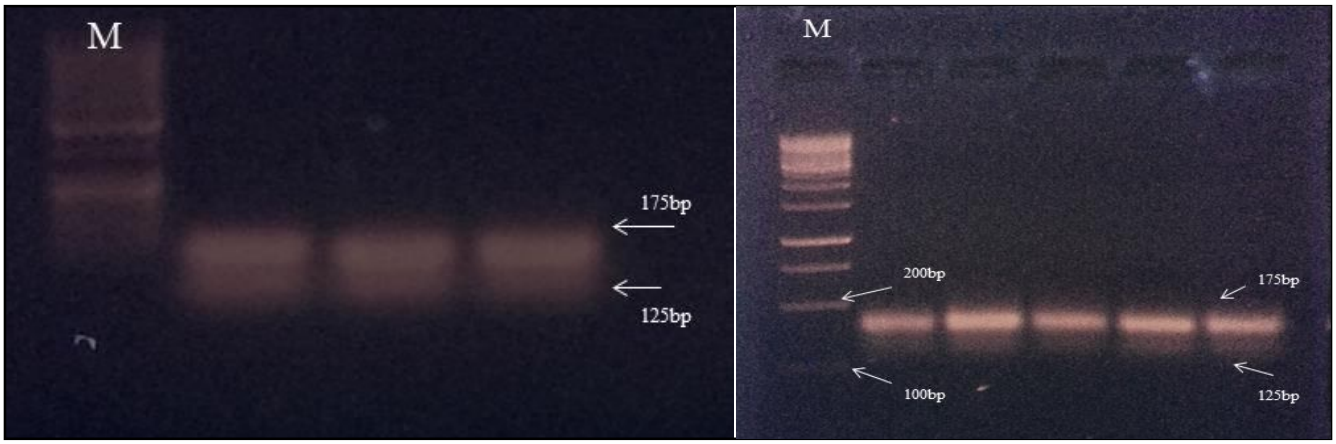

Fig. 2: Gel electrophoresis of LTF gene. PCR product after digestion with EcoR1 restriction enzyme, two alleles are resulted. From left to right, lane-1 = molecular size marker; lane 2-5 = two alleles, A (125) bp; B (175) bp.

AA and AB of LTF gene with frequency 57.14% and 42.86% for AA and AB respectively. And claimed there is no significant difference between bovine LTF gene polymorphism and SCC of milk (Šrubaøová *et al.*, 2009). Here in this study the AA, AB genotypes we found in Iraqi bovine LTF gene may be associated with SCC, therefore, LTF gene is highly responsible for increase resistant to mastitis in Iraqi cows which represents a serious danger that we must pay more attention to reduce incidences of occurrence. Further study including SCC investigation will improve our findings.

Conclusion

In summary, we showed that dairy cattle LTF gene polymorphism is distinguishable by examining LTF/*Eco*RI, and this gene could be as a marker for susceptibility/ resistance to mastitis. Our results also revealed that Iraqi cows are genetically stable with polymorphism which make them more resistance to infection. We suggest including LTF gene in the selection future program to improve immunity in our local dairy cows.

Acknowledgments

I specially thank Dr. Maytham.A. Dragh and my university. I also thank the (College of Life science, Misan University).

Author contributions

Z.S.A., designed the study. Z.S.A., performed all experiments in the study under the guidance of Z.S.A., analyzed and interpreted the data. Z.S.A., provided advice regarding experiments and writing of the manuscript. Z.S.A., wrote the manuscript.

Competing financial interests

The author declare no competing financial interests.

References

K. Wojdak-Maksymiec, M. Kmice and J. Ziemak (2006). *Vet. Med -Czech.*, **51:** 14-20.

G. Sender, A. Korwin-Kossakowska, K.G.A. Hameid and B. Prusak (2006). *Medycyna Wet.*, **62:** 563-565.

P. Šrubaøová, J. Dvoøák, International Ph.D. Students Conference (2009). Czech Republic, Brno, (MZLU v Brnì, Brno, 2009), 148.

Rainard, P. and C. Riollet (2005). Innate immunity of the bovine mammary gland. *Vet. Res.*, **37:** 369-400.

Chen, P. and F.C. Mao (2004). Detection of lactoferrin in bovine and goat milk by enzyme-linked immunosorbent assay. *J. Food Drug Anal.*, **12(2):** 133-139.

Ashwell, M.S., D.W. Heyen, T.S. Sonstegard, C.P. Van Tassell, Y. Da, P.M. VanRaden, M. Ron, J.I. Weller and H.A. Lewin (2004). Detection of quantitative trait loci affecting milk production, health and reproductive traits in Holstein cattle. *J. Dairy Sci.*, **87(2):** 468-475.

Kolbehdari, D., Z. Wang, J.R. Grant, B. Murdoch, A. Prasad, Z. Xiu, E. Marques, P. Stothard and S.S. Moore (2009). A whole genome scan to map QTL for milk production traits and somatic cell score in Canadian Holstein bulls. *J. Anim. Breed Genet.*, **126(3):** 216-227.

Kaminski, S., K. Oleñski, P. Brym, T. Malewski and A.A. Sazanov (2006). Single nucleotide polymorphism in the promoter region of the lactoferrin gene and its associations with milk performance traits in Polish Holstein-Friesian cows. *Russ. J. Genet.*, **42(8):** 924-927.

O'Halloran, F., D.P. Berry, B. Bahar, D.J. Howard, T. Sweeney and L. Giblin (2010). Polymorphisms in the bovine lactoferrin promoter are associated with reproductive performance and somatic cell count. *J. Dairy Sci.*, **93(3):** 1253-1259.

Sambrook, J. and D.W. Russell, Translated by Huang Pei Tang (2002). Molecular Cloning: A Laboratory Manual. 3rd. Science Press, Beijing, China.