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GENETIC VARIATION ANALYSIS OF ATPASE GENE AND ITS ASSOCIATION WITH MILK COMPONENTS IN CATTLE

Jaafar M. Owaid[1]Asaad Y. Ayied[1]&Faiza A. Ahmed[2]

1Research scholar, Animal Production Department, College of Agriculture, [2] Biology Department, College of Pure Science, University, Basrah, Iraq

ABSTRACT

There are huge number of cattle breeds or types are present all over the world and employed in different activities. The present study aimed to identify genetic variations and SNPs in mtDNA ATP6/8; among Holstein, Local Iraqi cattle and their crosses. The primer used in this study amplified 929-bp fragments from ATP6/8 gene. The results showed the presence of 8, 1 and 13 polymorphic sites leading to the construction of 5, 2 and 2 different haplotypes for Holstein, local and crosses respectively. Haplotype and nucleotide diversity were 0.576, 0.500 and 0.4786 and 0.00164, 0.00062 and 0.00763 respectively. Neighbor-joining trees were constructed using 34 samples showed that all studied cattle appeared into haplotype 1(H1), while Holstein also appeared in H4, H5, H6 and H7. Local breed included in H2 and the crosses in H3. AMOVA showed that variation within breed (between individuals was higher (85.83%) than between breeds (14.17%). Neutrality test both Tajim's D and Fu's Fs revealed that Holstein recorded highest negative values (-1.98343 and -1.18604 respectively). Whereas, the crosses cattle recorded positive values (0.91273 and 6.95086 respectively). The local breed showed positive and negative values near to zero (-0.61237 and 0.17185 respectively). Different haplotypes within Holstein breed associated significantly with fat%, lactose% and SNF%. Haplotypes 5 and 7 recorded highest percentages of fat, lactose and SNF when compared to other haplotypes. However, there were no such association within local or cross cattle. In conclusion, the identification of genetic variations and SNPs in cattle mitochondrial genes like ATP6/8 agene is of great interest because it has significant association with play important milk components and it can be recognized as a genetic marker for milk yield in cattle.

KEYWORDS:*Cattle, Atpase, Mtdna, Milk Components*

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INTRODUCTION

Mitochondrial DNA (mtDNA) is a powerful tool that can be used to determine evolutionary relationships, population composition and biology of many species due to its low molecular weight properties, simple structure, low recombination rate and rapid evolution rate (Curole and Kocher, 1999; Wan *et al*., 2004; Arif and Khan, 2009; Patwardhan et al., 2014; Hussain *et al*., 2015).

Among 15 different ATPase protein subunits in cattle (Anderson et al, 1982), there are eight subunits also called A6L and six subunits coded by mitochondrial ATPase 8 and 6 respectively (Witting and Schagger, 2008). Mutations in

ATPase lead to increase in ATP-TO-ADP level in mice (Eipel et al, 2011). As well as Yu et al, (2009a), Yu et al (2009b) and Weiss et al, (2012) indicated that variation in ATPase 8 lead to increase in mitochondria number and loss of endomatrix structure of renal cell mitochondria. Whereas, ATPase 6 is a subunit in mitochondria FiF0-ATP. It is a polypeptide of mitochondria endo membrane. Both ATPase 6 and 8 code in all eukaryotic creatures (Dewey et al, 1985). The length of ATPase 6 in cattle is 681bp, coded to 223 amino acids and it is the main part of proton channel (Bao et al, 2008). In mammals, ATP synthase contribute to oxidative phosphorylation, it contains not less than 16 subunits as well as ATP8 and ATP6 (Nijtmans et al, 1995 and Pederson et al, 2000).

Those two genes affect directly ATP synthesis and energy metabolism and may affect economical traits associated with metabolism as milk and fat yield in dairy cattle besides their use in evaluation of genetic polymorphisms among and within species (Avise, 2000; Barraclough, 2001; Perdices, 2001; Wong, 2004; Faulks, 2008; Hussain, 2015).

The objectives of the present study were to estimate genetic variation caused by ATPase8/6 among different breeds of cattle in Iraq and its association with milk chemical contents.

MATERIALS AND METHODS

The study was conducted for the period from 01/04/2017 to 01/08/2018, at the laboratory of Genetic Engineering at the University of Basrah, following with the collection of data from the field up to 15/03/2018. The study included the use of 40 cows (20 Holstein, 10 local and 10 crosses). The blood samples (5ml/cow) from the jugular vein were collected. Milk chemical contents included fat%, protein%, lactose% and solid not fat (SNF%) were estimated by Lactoflash produced by Funke Gerber, Germany. Samples of milk (50 ml) were collected every 15 days throughout the experiment period.

The analyses were carried out on 40cows. Blood from each cow was sampled intravitally into sterile vacuum tubes containing K_2EDTA (dipotassium ethylene diamine tetra acetic acid) anticoagulant. A fragment (929 Pb) of the mtDNA ATPase8/6 in the reference cattle mitochondrial genome by using the primer F- GCT ATA TAG CAC TAA CCT TTT و R- GCT TGG GTT TAC TAT ATG A (Vakalounakis and Fragkiadakis, 1999). The PCR amplifications were conducted in a 50 µl volume containing 20 ng genomic DNA, 25 µl of Master Mix, 2 µl each primer, 15 µl free water. The amplification conditions were as follows: initial denaturation at 94 C for 2 min followed by 35 cycles of denaturation at 94 C for 0.5 min, annealing at 58 C for 0.5min, and extension at 72 C for 0.5 min, and then the final extension at 72 C for 10 min. The PCR products were electrophoresed on 2% agarose gel stained with ethidium bromide to test the amplification success. The amplified products were purified with a DNA purification kit (SSufine) according to the manufacturer's instructions to remove residual primers and dNTPs. Sequencing was performed in sync ™ DNA Extraction Kit was used for DNA extraction and manufactured by the Taiwanese Geneaid company.

DATA ANALYSIS

ATPase8/6 sequences were aligned using the BioEdit software (Hall, 1999). Haplotype diversity (HD) and nucleotide diversity (π) were analyzed using DnaSP v5. 10 software (Librado and Rozas, 2009). Genetic distance, molecular variation (AMOVA) and neutrality test were analyzed using Arlequin 3.5.1.2 software (Excoffier and Lischer, 2010). The haplotypes network was drawn using Network 5.0.0.0 software (Bandelt et al., 1999). Neighbor-joining (NJ) tree for testing cattle breed sequences and the phylogenetic tree the three genotypes (Holstein, Local and crosses) were constructed using Megaversion 7.0 software (Kumar *et al*, 2016).

STATISTICAL ANALYSIS

The complete random design (CRD) completely Randomized design was used to analyze the data on the productive qualities studied using the SPSS (2012) Statistical program Version 22 and compared the averages using the General Linear Model within the program. The model included two factors, the first was the breed (Holstein, local and their crosses) and the second was the haplotypes within breed.

RESULTS AND DISCUSSION

Genetic Diversity

Number of ATPase8/6 sequences were 23 (table, 1). Seven, eight and nineteen of sequences belonged to local cattle, cross and Holstein respectively. Haplotypes number (H) were nine distributed as 2 for local, 2 for crosses and 5 for Holstein. Local, crosses and Holstein cattle showed a polymorphisms (NH) of 1, 13 and 8 respectively. Holstein revealed the highest value of haplotype diversity (HD) (0.576), followed by the local breed (0.500) and the cross cattle (0.476). On contrary, cross cattle recorded highest nucleotide diversity (π) followed by Holstein and local cattle (0.00763, 0.00164 and 0.00062 respectively).

Table 1: Genetic Diversity of Atpase8/6 Gene Among Different Cattle Breeds

Breeds	Number Of Sequences (N)	Haplotype Number (H)	Number Of Polymorphisms (NH)	Haplotype Diversity (HD)	Nucleotide Diversity
Local	7	2	1	0.5	0.0006
Crosses	8	2	13	0.476	0.0076
Holstein	19	5	8	0.576	0.0016

Haplotype Network

A total number of haplotypes of ATPase8/6 gene showed by different breeds were seven (fig. 1). The central circle represents Haplotype 1 (H1). Six branches appeared from H1, the first branch represents H2 which differed from H1 by one base (239) and included the local cattle only. The other branch represented H3 shown by one cross cow and differed from H1 by 13 bases. Whereas, the haplotypes H4, H5, H6 and H7 represented the Holstein cattle and differed from H1 by 4, 1, 1 and 2 bases respectively.

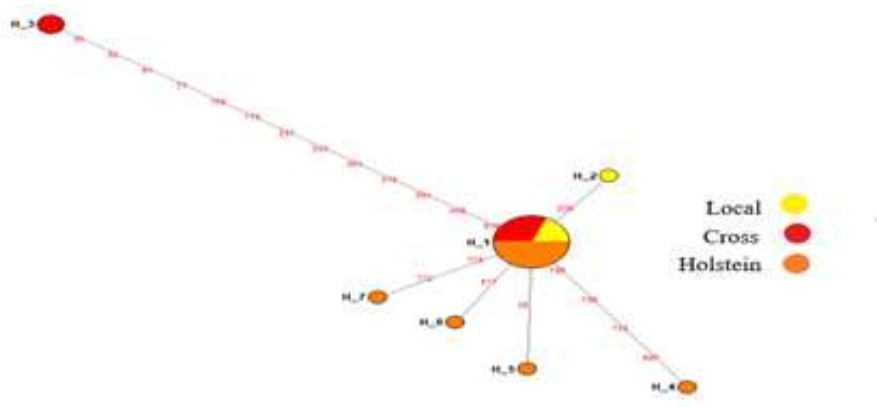

Fig. (1) Haplotype network of ATPase 8/6 gene among studied cattle

Analysis of Molecular Variation (AMOVA)

AMOVA of ATPase 8/6 gene among studied breeds of cattle resulted in between breed variation of 14.17% and within breed variation of 85.83% (table, 2).

Table 2: Analysis of Molecular Variance (AMOVA) of Atpase 8/6 Gene for Studied Breeds

Source of Variation	Degree of Freedom	Sum Squares	Variance Components	Variation %
Between Breeds	2	5.792	0.22251	14.17
Within Breeds	31	26.958	1.34791	85.83
Total	33	32.750	1.57042	

Neutrality Test

The results of neutrality test (Tajima's D) of the ATPase 8/6 gene (table, 3) showed negative values for local breeds (-0.61237) and Holstein (-1.98343), but it was positive (0.91273) for cross cows. Those of Fu's Fs were 0.17185, 6.95086 and -1.18604 for local, cross and Holstein cattle respectively.

Table 3: Neutrality Test of Atpase 8/6 Gene for Studied Cattle Breeds

Breeds	Tajima's Test (D)	Fu's Fs Test
Local	-0.61237	0.17185
Cross	0.91273	6.95086
Holstein	-1.98343	-1.18604

Atpase Polymorphisms And Milk Chemical Contents

Overall mean of fat%, protein%, lactose% and total solid not fat (SNF%) of Holstein were 2.99%, 4.52%, 5.07% and 9.09% respectively (table, 4). Those of cross cows were 3.74%, 3.03%, 4.64% and 8.23% respectively. Whereas, their values for local breed were 3.76%, 4.47%, 6.25 and 8.16% respectively. Fat, protein and lactose proportions were significantly (P<0.05) affected by breed. Local breed significantly (P< 0.05) exceeded those of Holstein and cross cattle. However, Holstein and cross cattle recorded higher (P<0.05) protein% than local breed.

Table 4: Milk Chemical Contents (%) of Different Breeds

Breeds	Fat	Protein	Lactose	SNF
Local	8.16±0.49 B	6.25±0.38 A	0.14±4.47	0.18±. 3.76
Cross	9.09 ±0.77 A	5.07±0.38 B	. 0.18±303B	0.72±3.74A
Holstein	9.09±0.77 A	5.07±0.38 B	0.19±4.52A	0.58±2.99B

Different haplotype associated significantly (P<0.05) with fat%, protein%, lactose% and SNF% (table, 5). H5 exceeded significantly (P<0.05) other haplotypes in fat%, lactose% and SNF% (4.55%, 5.73% and 9.38% respectively) except H7 revealed similar fat% (4.94%), H2 produced similar lactose (5.36%) and H4 produced similar SNF (9.17%).However, H5 recorded the least value of protein (3.65%).

Table 5: Association of Atpase 8/6 Haplotypes and Milk Chemical Components

Haplotypes**	Fat	Protein	Lactose	SNF
H1	3.44±0.04c	4.02±0.01a	4.96±0.03b	8.15±0.03 b
H2	3.65±0.06 b	4.08±0.08 a	5.36±0.16 a	7.44±0.20 c
H3	3.28±0.22 c	3.93±0.08 a	4.92±0.16 b	8.22±021 b
H4	3.88±0.22 b	4.00±0.07 a	5.08±0.15 b	9.17±0.19 a
H5	4.55±0.23 a	3.65±0.08 b	5.73±0.16 a	9.38±0.19 a
H6	3.89±0.28 b	4.02±0.07 a	4.78±0.15 b	8.33±0.20 b
H7	4.94±0.21 a	3.90±0.08 a	4.97±0.16 b	8.77±0.19 b

Means with different subscripts differ significantly (P<0.05) within the same column

Means were adjusted from the mean of breed.

DISCUSSION

The results of the present study showed that polymorphism of cross breed reflecting that the source of genetic variation is the crossing processes or migration. These results are in agreement with those of Dadi et al (2009) in Ethiopian cross cattle. However, local breed exhibited low polymorphism which indicated that genetic content of this breed has not changed and the main factor changing the genetic variation is the genetic drift as a result of holding this breed in very small herds (not more than 3 cows/breeder). As well as this breed distributed in remote areas and kept by breeder using very old not scientific method of breeding inherited from their ancestors. These practices elevated inbreeding which increases homozygosity and genotypic fixation. Especially it showed clear reduction in nucleotide diversity of the studied gene and revealed clear genetic differences from both the Holstein and their crosses. Besides crossing local breed with Holstein is not under systematic crossing program with no clear target. Wang et al (2018) concluded that ATPase 8/6 associated with high altitude adaptation cattle. In case of local and crossed cattle diversity results expressed as polymorphisms and haplotype number were lower than those recorded by Qin et al (2012), however, result of Holstein was similar.

High haplotype and nucleotide diversity of Holstein breed (0.563 and 0.00609) encourage the use of this gene as molecular marker for milk quality. The positive results of this gene are in agreement with beef studies using same gene or D-Loop (Mannen et al, 2003 and Zhang et al, 2008).

REFERENCES

1. *Bandelt HJ, Forster P and Rohl A, (1999). Median-Joining Networks for inferring intraspecific phylogenies. Mol. Biol. Evol., 16(1): 37-48.*
2. *Curole J. P. and KocherT. D. (1999). Mitogenomics: digging deeper withcomplete mitochondrial genomes. Trends in Ecology & Evolution 14(10):394-398. DOI: 10.1016/S0169-5347(99)01660-2*
3. *Chung, H. (2013). Phylogenetic analysis and characterization of mitochondrial DNA for Korean native cattle. Open Journal of Genetics 3(01): 12.*
4. *Cortes, O., Tupac□Yupanqui, I., Dunner, S., García□Atance, M., García, D., Fernández, J. &Cañón, J. (2008). Ancestral matrilineages and mitochondrial DNA diversity of the Lidia cattle breed. Animal Genetics 39(6): 649-654.*
5. *Excoffier L and Lischer HE, (2010). Arlequin suite ver. 3.5: a new series of programs to perform population genetics analyses under Linux and Windows. Molecular Ecology Resources 10 (3): 564-567.*
6. *Hall TA, (1999). BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucleic Acids Symposium, 41: 95–98.*
7. *Kantanen, J., Edwards, C. J., Bradley, D., Viinalass, H., Thessler, S., Ivanova, Z., Kiselyova, T., Ćinkulov, M., Popov, R. &Stojanović, S. (2009). Maternal and paternal genealogy of Eurasian taurine cattle (Bos taurus). Heredity 103(5): 404.*

8. *Kumar S, Stecher, G and Tamura K, (2016). MEGA7: Molecular Evolutionary Genetics Analysis Version 7.0 for bigger datasets. Molecular Biology and Evolution 33 (7): 1870-1874.*

9. *Lei, C., Chen, H., Zhang, H., Cai, X., Liu, R., Luo, L., Wang, C., Zhang, W., Ge, Q. & Zhang, R. (2006). Origin and phylogeographical structure of Chinese cattle. Animal Genetics 37(6): 579-582.*

10. *Librado P and Rozas J, (2009). DnaSP v5: A software for comprehensive analysis of DNA polymorphism data. Bioinformatics, 25 (11): 1451–1452.*

11. *Loftus, R., Hugh, D. M., Ngere, L., Balain, D., Badi, A., Bradley, D. & Cunningham, E. (1994). Mitochondrial genetic variation in European, African and Indian cattle populations. Animal Genetics 25(4): 265-271.*

12. *Mannen, H., Morimoto, M., Oyama, K., Mukai, F. & Tsuji, S. (2003). Identification of mitochondrial DNA substitutions related to meat quality in Japanese Black cattle. Journal of animal science 81(1): 68-73.*

13. *Qin, Y.H., Chen, S.Y. & Lai, S.J. (2012). Polymorphisms of mitochondrial ATPase 8/6 genes and association with milk production traits in Holstein cows. Animal biotechnology 23(3): 204-212.*

14. *Romaino, S., Fazly-Ann, Z., Loo, S., Hafiz, M., Hafiz, M., Iswadi, M., Kashiani, P., Rosli, M., Syed-Shabthar, S. & Md-Zain, B. (2014). Species identification of Malayan Gaur, Kedah-Kelantan and Bali cattle using polymerase chain reaction-restricted fragment length polymorphism. Genet. Mol. Res 13(1): 406-414.*

15. *Salim, B., Taha, K. M., Hanotte, O. & Mwacharo, J. M. (2014). Historical demographic profiles and genetic variation of the East African Butana and Kenana indigenous dairy zebu cattle. Animal genetics 45(6): 782-790.*

16. *Sutarno A. &Setyawan D.(2015). Review: Genetic diversity of local and exotic cattle and their crossbreeding impact on the quality of Indonesian cattle. Biodiversitas: 16:327-354.*

17. *Tarekegn, G. M., Ji, X.-y., Bai, X., Liu, B., Zhang, W., Birungi, J., Djikeng, A. & Tesfaye, K. (2018). Variations in mitochondrial cytochrome b region among Ethiopian indigenous cattle populations assert Bos taurus maternal origin and historical dynamics. Asian-Australasian journal of animal sciences, 31(9):1393-1400. Doi: 10.5713/ajas.17.0596.*

18. *Wang, J., Shi, Y., Elzo, M. A., Dang, S., Jia, X. & Lai, S. (2018). Genetic diversity of ATP8 and ATP6 genes is associated with high-altitude adaptation in yak. Mitochondrial DNA Part A 29(3): 385-393.*

19. *Zhang, B., Chen, H., Hua, L., Zhang, C., Kang, X., Wang, X., Pan, C., Lan, X. & Lei, C. (2008). Novel SNPs of the mtDNA ND5 gene and their associations with several growth traits in the Nanyang cattle breed. Biochemical genetics 46(5-6): 362-368.*