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Detection of SNPs in FABP4 Gene and Its Relationship with Milk Quality Traits in Iraqi Jenoubi Cows

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Abstract: Jenoubi cattle breed classified as a *Bos indicus* L., is one of Iraqi indigenous cattle that kept for milk production purpose, but the influence of fatty acid-binding protein 4 (FABP4) on milk-related traits remain poorly understood. The current study was conducted to investigate the effect of single nucleotide polymorphisms (SNPs) in the FABP4 gene of Jenoubi cows. The DNA samples were extracted from the blood of 21 cows. Along 565-bp nucleotide (nt) amplicon from the intron 2- exon 3- intron 3 of FABP4 was amplified by polymerase chain reaction (PCR) while forward sequencing was used to detect SNPs and results were analyzed using bioinformatics software. The findings resulted the existence of two SNPs (c.3689G>A and c.3709G>C) in the exon 3 and four SNPs (g.3494A>C, g.3531A>T, g.3743T>C and g.3765T>C) in the intron regions. Therefore, three haplotypes of *FABP4* gene were obtained in this study based on three SNPs of g.3494A>C, g.3531A>T and g.3765T>C. Nonetheless, those haplotypes were not significantly influencing to the milk quality traits of Jenoubi cows. However, the SNPs examined in this study might not be used as potential DNA marker to improve milk production traits of the current breed.

Keywords: *FABP4* gene, Iraqi Jenoubi Cows, Milk yield, Single nucleotide polymorphism.

Introduction

Cattle raising for milk and meat production is a significant element of the economy in Iraq's rural areas, contributing significantly to agricultural revenue and as well as producing more than half of the nation's animal products (Faraj *et al.*, 2020a). Iraqi Jenoubi cattle are scattered across the country southern. They are said to be descended from Indian cows (Zebu), which have comparable traits to the species (*Bos indicus*) that inhabit hotter climates; nonetheless, they differ from European cattle belonging to the *Bos Taurus* (Faraj *et al.*, 2020b). Characteristics for milk production are critical. Understanding the genetic basis of milk production features is crucial in animal production and the linked economy (Faraj *et al.*, 2019). The techniques of

molecular genetics, which allow for the finding of genes that have a substantial impact on complex characteristics, are one strategy that has gained favor (Zhou *et al.*, 2015).

At the moment, molecular breeding technologies such as marker-assisted selection (MAS) and genomic selection breeding are being gradually used in animal breeding (Chen *et al.*, 2018). As a result, identifying the genetic variants and functional genes associated with cow's milk production features is essential (Dekkers, 2004). *FABP4* gene is one of the members of the *FABP* family (*FABP1-FABP9*) they are also expressed in various tissues. The gene is located on chromosome 14 along 4,389 bp (46,833, 665-46, 838,053) that rich in QTL for milk production traits (Khatkar *et al.*, 2004). The bovine *FABP4* gene consisted of 4 exons and 3 introns (Nafikov *et al.*, 2013). The main function of *FABP4* is to bind and transport long-chain fatty acids (LCFAs) within animal cells, including in the mammary gland cells (Kulig *et al.*, 2013). Gene expression of the *FABP4* is greatly up-regulated during lactation (Bionaz & Loor, 2008).

Previous studies reported that *FABP4* gene was significantly associated with back fat thickness of Korean bulls (Cho *et al.*, 2008), fatty acid composition in Japanese Black cattle (Hoashi *et al.*, 2008), Korean bulls (Oh *et al.*, 2012), marbling score and carcass weight of Korean bulls (Lee *et al.*, 2010), carcass characteristic of Turkish Holstein bulls (Ardicli *et al.*, 2017) and Nellore cattle (Curi *et al.*, 2011), milk production of Jersey cows (Kulig *et al.*, 2013), meat chemical composition of cull Aceh cows (Al-Azhar *et al.*, 2020), growth traits of Nellore cattle (Ayres *et al.*, 2010), meat quality of Yanbian yellow bulls (Yin *et al.*, 2020), milk production and milk quality of Holstein × Jersey cows (Zhou *et al.*, 2015; Li *et al.*, 2019), Jersey, Piedmontese and Valdostana (Marchitelli *et al.*, 2013). Hence, it can be suggested that the *FABP4* gene may be a milk-producing candidate gene or marker in Jenoubi cows. The purpose of the present study was to investigate the association between SNPs of *FABP4* gene and milk qualty traits of Iraqi Jenoubi cows. The results in this study can be used to improve milk quality traits of Iraqi Jenoubi cows through the molecular selection program.

Materials & Methods

Sampling and DNA extraction

This study was carried out in the Molecular Biology laboratory in the Department of Biology, College of Science, University of Misan, Iraq. Milk samples were collected in a 50 ml tube and sent to the physiology laboratory (College of Agriculture, University of Basrah) for milk analysis. The blood samples were collected randomly from 21 Iraqi Jenoubi Cows. The bloods samples (5 ml/cow) were collected from the jugular vein using venoject needle and vacutainer tubes containing EDTA. The genomic DNA was extracted with DNA Extraction Kit (Geneaid, Taiwan). The target sequence of *FABP4* gene along 565 bp (intron 2, exon 3 and intron 3) was determined using the primer Forward: 5`-ACCCCTATGATGCTATTCCACA-3`and Reverse:5`-ATACGGTTCACATTGAGAGGGA-3` (Shin *et al.,* 2012) as shown in fig. (1).

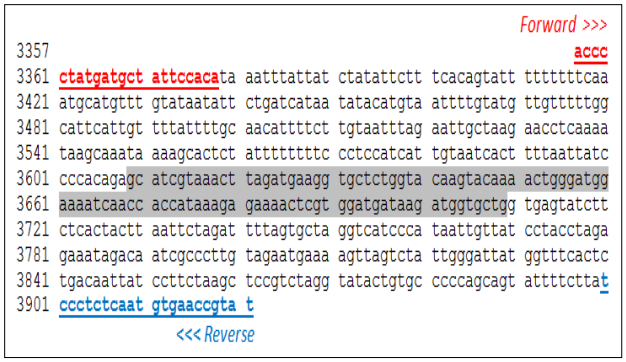

Fig. (1): The target sequence of *FABP4* gene along 565 bp based on GenBank: NC_037341.1. The grey bold is an exon 3 region.

The PCR amplifications were conducted in a 25µl^{-1} volume containing approximately 3 µl^{-1} genomic DNA, 12.5 µl^{-1} of Master Mix, 2 µl^{-1} of forward/reverse primers, and 7.5 µl^{-1} free water. The PCR amplification protocol conditions: pre-denaturation at 95°C for 4 min followed by 35 cycles of denaturation stage at 94°C for 45s, annealing at 59° C for 1 min, and extension at 72° C for 1.5 min, followed by the final extension stage at 72° C for 6 min. The PCR product was detected by 1% agarose gel electrophoresis and sent to the Macrogen Company (South Korea) for the forward sequencing analysis.

Statistical analysis

The genetic diversity parameters such as genotype frequency, allele frequency were calculated Takezaki *et al.* (2010), expected and observed heterozygosity (He) and observed heterozygosity (Ho) (Weir, 1991). Polymorphism informative content / PIC was calculated referring to Botstein *et al.* (1980), Hardy-Weinberg equilibrium (HWE) and Chi-square test referring to Kaps & Lamberson, (2004) and number of effective allele / n_e (Nei & Tajima, 1981) were calculated using Microsoft Office Excel 2007 program. Therefore, the linkage disequilibrium. r-^{2} (Hui & Burt, 2020) was calculated using a similar program. In addition, the association analysis between *FABP4* gene polymorphism and milk quality of Iraqi Jenoubi cow's population were computed with a randomized block design model as follows:

$$Yik = \mu + Gi + ek$$

where, Yik is the observed variable, µ is the overall means of traits, Gi is the fixed effect of ith genotype and ek is the random residual effect.

Results

Along 565 bp of the *FABP4* gene in animal studies was successfully amplified in 1% of agarose gel as shown in fig. (2).

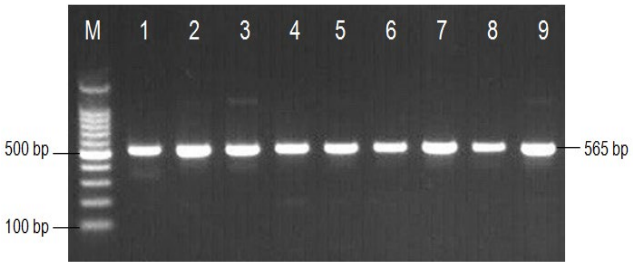

Fig. (2). The amplification of *FABP4* gene in Iraqi Jenoubi cattle along 565 bp in 1% of agarose gel. M: DNA ladder 100 bp. Line 1 - 9: DNA sample

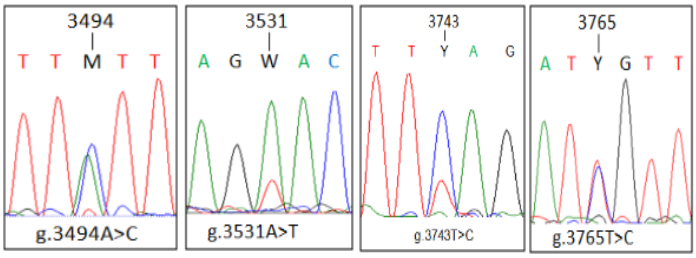

Fig. 3. Four common SNPs at intron 2 of *FABP4* gene in Iraqi Jenoubi cows. M: A/C; W: A/T; Y: C/T

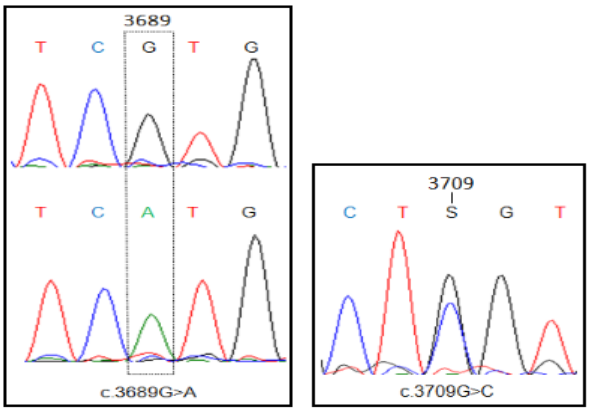

Fig. 4. Two common SNPs in the exon 3 of *FABP4* gene in Iraqi Jenoubi cows. S: G/C

A total of six (6) SNPs were detected in the *FABP4* gene of animal studies as shown in Table 1. Two SNPs were located in exon 3 region (c.3689G>A; c.3709G>C) and the other SNPs located in intron 2 region (g.3494A>C; g.3531A>T) and intron 3 (g.3743T>C; g.3765T>C).

The chromatogram in the SNPs at non-coding and exon 3 regions of FABP gene in animal studies were illustrated in fig. (3) and fig. (4), respectively.

In this study, three mutation sites in the linkage SNP have the moderate genetic diversity (0.30<PIC<0.50) as shown in table (2).

In contrast, two SNPs in the exon 3 of *FABP4* gene of animal studies has low genetic diversity (PIC<0.30) as shown in table (3).

Table (1): Six (6) common SNP's in *FABP4* gene of Iraqi Jenoubi cows based on the sequencing and alignment results

SNP	Position	Mutation	N	Type	Region	AA change
1	3494	A → C	14	Transversion	Intron 2	-
2	3531	A → T	7	Transversion	Intron 2	-
3	3689	G → A	2	Transition	Exon 3	Val - Met
4	3709	G → C	2	Transversion	Exon 3	Synonymous (Leu)
5	3743	T → C	2	Transition	Intron 3	-
6	3765	T → C	14	Transition	Intron 3	-

Table (2): Genetic diversity in the linkage SNP of *FABP4* gene in Iraqi Jenoubi cows

Haplotype (SNP1/SNP2/SNP6)	Frequency (N)	Allelic frequency		H_o	H_e	n_e	PIC	χ^2
		A/A/T	C/T/C					
AA/AA/TT	0.10 (2)							
AC/AT/TC	0.57(12)	0.38	0.62	0.57	0.42	1.89	0.36	0.94*
CC/TT/CC	0.33 (7)							

Table (3): Genetic diversity in the non-linkage SNP of *FABP4* gene in Iraqi Jenoubi cows

SNP	Genotypic frequency (N)		Allelic frequency		H_o	H_e	n_e	PIC	χ^2
3	GG (19)	AA (2)	G (0.91)	A (0.09)	0.00	0.17	1.21	0.16	21.00
4	GG (18)	CG (3)	G (0.93)	C (0.07)	0.14	0.13	1.15	0.12	0.12*
5	TT (18)	TC (3)	T (0.93)	C (0.07)	0.14	0.13	1.15	0.12	0.12*

N: number of observations; H_o: observed heterozygosity; H_e: expected heterozygosity; n_e: number of effective alleles; PIC: Polymorphic informative content; χ^2: Chi-square; *under Hardy-Weinberg equilibrium

The linkage disequilibrium (r^2) value in *FABP4* gene of animal studies was ranged from 0.105 (SNP5 - SNP6) to 0.539 (SNP1 - SNP6) as shown in table (4).

In this study, the effect of linkage SNP (haplotype) was not affected to the milk quality traits in Jenoubi cows as shown in table (5). However, animals with haplotype CC/TT/CC have the highest fat and lactose percentages rather than other haplotypes. Meanwhile, the highest of protein content was reached by animals with haplotype AC/AT/TC (heterogeneous haplotype).

Table (4): Linkage disequilibrium (r^2) in the *FABP4* gene of Iraqi Jenoubi cows

r2	SNP2	SNP3	SNP4	SNP5	SNP6
SNP1	0.222	0.475	0.148	0.148	0.539
SNP2		0.107	0.107	0.107	0.218
SNP3			0.051	0.051	0.420
SNP4				0.823	0.105
SNP5					0.105

Table (5): Effect of different haplotype to milk quality in Iraqi Jenoubi cows

Haplotype	Fat (%)	Protein (%)	Lactose (%)
AA/AA/TT	5.74±0.35 (2)	3.83±0.06 (2)	4.79±0.33 (2)
AC/AT/TC	5.48±0.54 (12)	4.00±0.11 (12)	4.99±0.19 (12)
CC/TT/CC	5.84±0.58 (7)	3.95±0.22 (7)	5.06±0.13 (7)

Discussion

Yin *et al.* (2020) obtained the similar SNPs in Chinese Yanbian Yellow (CYY) cattle that significantly associated with meat quality traits. Numerous earlier investigations found that SNP3 (c.3689G>A) in Korean (Cho *et al.*, 2008) and Japanese Black (Hoashi *et al.*, 2008) cattle, had no effect on carcass characteristics. Meanwhile, SNP3 was significantly associated with marbling score in Hanwoo (Lee *et al.*, 2010) and Holstein bulls (Ardicli *et al.*, 2017), milk fatty acid levels in Holstein × Jersey cows (Li *et al.*, 2019) and saturated fatty acid in Jersey, Piedmontese and Valdostana cows (Marchitelli *et al.*, 2013). Despite of SNP3, SNP4 (c.3709G>C) were significantly associated with fatty acid (C18:1) composition in Korean cattle (Oh *et al.*, 2012). Unfortunately, SNP3 and SNP4 can not be used as the molecular selection because of low genetic diversity (PIC<0.30). Goszczynsky *et al.* (2017) reported that SNP2 (g.3531A>T) and SNP3 were absence in Holstein, Wagyu, Brahman and Limousin cattle. Moreover, SNP3 was monomorphic in Aceh cattle of Indonesia (Al-Azhar *et al.*, 2020). Fathoni *et al.* (2019) reported that SNP4 in *FABP4* gene of Kebumen Ongole grade cattle can not be used as molecular selection because of low genetic diversity. In this study, the effect of haplotype (SNP1/SNP2/SNP6) did not affected the milk quality of Iraqi Jenoubi cows although has moderate PIC value. Interestingly, the haplotype of *FABP4* gene was under HWE equilibrium and

indicated that no selection that influencing haplotype diversity. Actually, a SNP with high PIC value can be used for molecular selection (Takezaki *et al.* 2010). Hence, the depth research with large sample is important to obtain the results accurately. Yin *et al.* (2020) obtained the linkage disequilibrium (r^2) value in *FABP4* gene of CYY cattle about 0.791 to 1.00. The r^2 value in the present study was lower than those in CYY cattle because of different genetical factor. In this study, the highest r^2 value was showed in SNP1 - SNP 6. Meanwhile, in CYY cattle the highest r^2 value was showed in SNP4 - SNP5 and SNP5 - SNP6. The linkage disequilibrium was affected by recombination, genetic drift, inbreeding, mutation and gene flow (Banos *et al.*, 2008).

Conclusion

The *FABP4* gene in Iraqi Jenoubi cows was polymorphic with presence of six (6) common SNP's. However, two SNP's at exon 3 have low PIC value. A haplotype of SNP1/SNP2/SNP6 was detected with moderate PIC value. Nevertheless, the obtained haplotype in animal studies did not affect the milk quality traits of Iraqi Jenoubi cows.

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Contributions of authors

S.H.F.: Blood and milk samples collection, laboratory methodology, and writing part of the manuscript.

W.P.B.P.: Suggest a title of the manuscript, statistical analysis and writing part of the manuscript.

T.L.T.: Evaluation and writing part of the manuscript.

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Conflicts of interest

Authors declared that there is no conflict of interests.

Ethical approval

All ethical guidelines related to animal care issued by national and international organizations were implemented in this report.

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الكشف عن تعدد أشكال النيوكليوتيدات المنفردة في جين *FABP4* وعلاقته بصفات جودة الحليب في الأبقار الجنوبية العراقية

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المستخلص: تصنف الابقار الجنوبية على انها *Bos Indicus*، وتعد من الابقار العراقية التي تربى لغرض انتاج الحليب، ولكن تأثير البروتين المرتبط بالأحماض الدهنية 4 (FABP4) على الصفات المرتبطة بالحليب لا يزال غير مفهوم جيداً. أجريت الدراسة الحالية لمعرفة تأثير تعدد اشكال النيوكليوتيدات المنفردة (SNPs) في الجين المشفر للبروتين الرابط للأحماض الدهنية (FABP4) على صفات جودة الحليب في الابقار الجنوبية العراقية. تم استخلاص الحامض النووي منقوص الاوكسجين (DNA) من عينات الدم لـ 21 بقرة. ضخمت قطعة بطول 565 زوج قاعدي في الانترون الثاني والاكسون الثالث والانترون الثالث لجين *FABP4* باستخدام تقانة تفاعل البلمرة المتسلسل (PCR) وكذلك استخدمت تقانة تتابع القواعد النيتروجينية للكشف عن التغيرات الوراثية وحللت النتائج باستخدام برامج المعلوماتية الحيوية. أظهرت النتائج الكشف عن وجود تشكلين وراثيين في النيوكليوتيدات المنفردة ((c.3689G>A and c.3709G<C في الاكسون الثالث وأربعة تشكلات للنيوكليوتيدات المنفردة (g.3494A>C, g.3531A>T, g.3743T>C and g.3765T>C) في مناطق الانترون. لذلك، تم الحصول على ثلاثة أنماط فردية من جين *FABP4* في هذه الدراسة بناءً على الاشكال الوراثية الاتية g.3494A> C و g.3531A> T و g.3765T> C. ومع ذلك فأن الأنماط الفردية التي تم الكشف عنها لم يكن لها تأثير على صفات جودت الحليب للأبقار الجنوبية.

الكلمات المفتاحية: جين *FABP4*، الابقار الجنوبية العراقية، تعدد أشكال النيوكليوتيدات المنفردة ومحصول الحليب.