ASSOCIATION OF LACTOFERRIN GENE POLYMORPHISM WITH MILK PRODUCTION AND COMPOSITION

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ABSTRACT

The objective of this study was to determine the genotypes and allele frequency of polymorphism at position +7605 in exon 4 of the goats lactoferrin gene and to quantify their association with milk production traits using 50 goats , A 430 bp product of Lactoferrin gene exon4 was amplified and the goats were genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method and *NlaIII* restriction enzyme. The study revealed of SNP (+7605 C/T) in Lactoferrin gene exon4 and Two alleles T and C, were identified in the studied population . Their frequencies were 0.77 and 0.23, respectively. The alleles controlled the occurrence of three genotypes: TT, TC and CC with frequencies 62 , 30 and 8 %, respectively. GLM (Generalized linear Model) analysis was applied to evaluate the association between lactoferrin gene genotypes and milk production and composition. Significant association was found (P<0.05) between SNP (+7605 C/T) genotypes and milk protein; animals with the CC genotype had higher mean values for milk protein than those with the TT ,TC genotype also the same genotype had significantly higher (P < 0.05) in milk production Compared with TT genotype . Other traits did not show any significant difference. the SNP has the potential to be considered as marker-assisted selection .

Key words: Goats , Lactoferrin gene , genotype , Milk production

العلاقة بين تعدد مظاهر جين اللاكتوفيرين مع انتاج الحليب وتركيبه

وفاء اسماعيل ابراهيم حمزة يوسف علي
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المستخلص

الهدف من الدراسة الحالية كان لتحديد التراكيب الوراثية والتكرار الاليلي عند الموقع 7605+ في جين اللاكتوفيرين للماعز وتحديد علاقته مع صفات انتاج الحليب باستعمال 50 معزة، تم تضخيم قطعة منتج مكونة من 430 زوج قاعدي لجين اللاكتوفيرين الاكسون الرابع حددت التراكيب الوراثية للمعزات باستعمال تقانة تعدد المظاهر لاطوال القطع المقيدة –PCR) RFLP) وانزيم التقييد *NlaIII*. كشفت الدراسة عن تعدد مظاهر في قاعدة مفردة SNP (+7605 C/ T) في جين اللاكتوفيرين اكسون 4 وعن اليلين T و C في العينة المدروسة وكانت تكرارها 0.77 و 0.23 بالتتابع وان هذه الاليلات تتحكم في ثلاث تراكيب وراثية TT وTC وCC وتكرارها 62 و30 و8 % بالتتابع. اعتمد تحليل النموذج الخطي العام (GLM) لتقييم العلاقة بين التراكيب الوراثية لجين اللاكتوفيرين وإنتاج الحليب وتركيبه. وجدت علاقة معنوية (P<0.05) بين التراكيب الوراثية SNP (+7605 C/ T) وبروتين الحليب إذ حققت الحيوانات الحاملة للتركيب الوراثي CC معدل اعلى من الحيوانات الحاملة للتراكيب TT وTC وأيضا نفس التركيب الوراثي CC له معدل انتاج حليب عالي ومعنوي (P<0.05) مقارنة مع التركيب الوراثي TT. الصفات الاخرى لم تظهر فروق معنوية، ان لتعدد المظاهر في نيوكليوتيدة مفردة SNP ممكن اعتبارها كمؤشر للمساعدة في الانتخاب.

كلمات مفتاحية : الماعز ، جين اللاكتوفيرين ، التركيب الوراثي ، انتاج الحليب .

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INTRODUCTION

Genetic variance in dairy performance traits in animals has been discovered for more than 20 years ago, giving much more than a few (QTLs) related to main economic traits. For example ABCG2, DGAT1 CASB, IGF2 and GHR and many other genes are among these exceptions but not without a contradiction in the results (13, 14). It has been shown many genes have strong relationship with health and immune system and growth traits, (11, 17). Lactoferrin is an iron-binding glycoprotein, is produced in many biological fluids in mammals, and closely related in structure to the transferrin (10). Its has been mentioned that lactoferrin have many physiological and antimicrobial functions . The study of lactoferrin structure demonstrated that there are binding sites for Fe^{+3} in its two clefts and it is best known for its ability to bind iron (4). Furthermore both bovine or human lactoferrin is an anabolic factor in skeletal tissue and a potent osteoblast survival factor (5). It stimulates the proliferation of bone-forming cells, osteoblasts, and cartilage cells , which further suggests that Lactoferrin has a physiological role in bone growth and a potential therapeutic role in osteoporosis (3, 6). Other functions of lactoferrin such as inhibiting tumor growth (8) and inhibiting liquid preoxidation (7) have also been reported lactoferrin is a potential genetic marker for mastitis resistance, because of his role in immune response during mastitis (16) , The most important advantage of lactoferrin gene and its promoter are highly polymorphic, many SNPs localized within the lactoferrin gene and its 5' regulatory regions have been demonstrated with effect on gene expression and resistance against pathogens (15). It was shown that lactoferrin SNPs are related to the somatic cell count (SCC) or Score (SCS) (12, 18), Since lactoferrin exhibits potential for further application as a mastitis resistance/susceptibility marker and selection for mastitis resistance cannot contradicts with selection for milk production traits there is a need to evaluate whether lactoferrin is associated with dairy traits in different animals , It is considered an important gene for milk production traits in all ruminants .The objective of the current study is to detect SNP (+7605 C/T) of the lactoferrin gene exon4 in dairy goats and explore their possible association with milk production traits in Iraqi goats.

MATERIAL AND METHODS

1.Samples Collection and DNA Extraction

Blood sample were collected from 50 goats in ruminants Researches station (20 km west of Baghdad) using vacuum tube needle and EDTA anticoagulants. and Samples were stored until extraction , The DNA was extracted using Geneaid extraction kit. The extraction was according to manufacturer company . All goats were kept in similar environmental conditions , Milk samples from each goat were collected and kept at -20°C. Milk compositions including milk fat, protein, solid-non-fat(SNF), and lactose were analyzed using (Milko Scan) . Milk production was measured every week for six months .

2.Polymerase Chain Reaction

According to the sequence of the goat lactoferrin gene (GeneBank accession No. NC_FJ609300),We designed primers to amplified 430 bp fragment, that includes exon 4 of goat lactoferrin gene Table 1 to determine the genotypes and alleles of the SNP (+7605 C/T) in goats lactoferrin gene exon4 , PCR reaction was preformed in size 20µL Master mix tube that contain 2 µL Forward primer and 2 µL Reverse primer and 4 µL genomic DNA and 12 µL distilled water . Amplification was carried out for 94^{o}C for 5 min; 35 cycles of 94 ^{0}C for 1 min, 56^{o}C for 1 min, 72oC for 1 min; and a final extension at 72^{o}C for 5 min.

Table1. designed Primers used to PCR Amplification

Forward primer	5-TGT CCC TGG GCT CTT TAG-3
Reverse primer	5-CCG AAG TGG CTT GTG AA-3

3.Restriction Fragment Length Polymorphism Analysis

Lactoferrin gene Polymorphism and genotypes were identified by PCR-RFLP method Samples ,10 µl of each PCR product , were incubated for 3.5 hours at 37 °C with 10 U *NlaIII* enzyme, according to manufacturer's instructions (Biolabs). Digestion products were separated by electrophoresis on 2 % agarose gel in 1×TBE buffer. The gels were run at 80 V for 1.5 hour and visualized under

ultraviolet light after staining with ethidium bromide.

4.Statistical analysis

Allele and genotype frequencies were calculated using Pop-Gene, 1.31 software (19) and. Statistical analysis was carried out by SAS 2012 (Statistical Analysis System) using general linear model (GLM) to test the association of different genotypes of the lactoferrin gene (+7605 SNP) and milk production and milk composition traits least squares means of the genotypes were compared to determine the best genotype Statistical model used in study :

$Y_{lkij} = A + \mu_i + B_j + G_k + e_{lkij}$

Y_{lkij} = Observation of any traits

μ= Overall mean

A_i=Fixed effect of age (2-4 year)

B_j = Fixed effect of breeds (Damascus, Local black)

G_k = Fixed effect of Lactoferrin Genotypes (TT,TC and CC)

e_{lkij} = Random error, which is distributed naturally at an average of zero and Variance $\sigma^2 e$

RESULTS AND DISCUSSION

1.Lactoferrin Gene polymorphism

Digestion of 430 bp fraqment of Lactoferrin gene exon 4 with *NlaIII* restriction enzyme revealed two alleles This is due to the missense mutation at +7605 position, The alleles were C and T. The recognition site of *NlaIII* enzyme is CATG at the wild sequence digestion of 430 bp product resulted two fragments 264 bp and 166 bp and mutant sequence the T base changed to C base and sequence Changed to CACG this will lead to absence of restriction site and 430 bp product remains at the same size . Three genotypes identified in this study TT genotype consists of two bands 266 bp and 164 bp the TC genotype consists of three bands 430bp, 264 bp and 166 bp the CC genotype Consists of one band 430bp, Figure1 show the digestion result and genotypes identified in the studied population , Akisa *et al*. (1) previously determined the SNP (+7605 C/T) of lactoferrin gene in Indigenous anatolian Goat breeds using PCR-RFLP and *NlaIII* enzyme and revealed on two alleles the wild type (T) and mutant type (C), Also the Current study disagree with Kang et al (9) that the SNP (+7605 C/T) can be determined by using *HindIII* restriction enzyme and also *HindIII* enzyme recognises the sequence CATG, the SNP (+7605 C/T) Can only be detected using *Hin1II* and *NlaIII* enzymes.

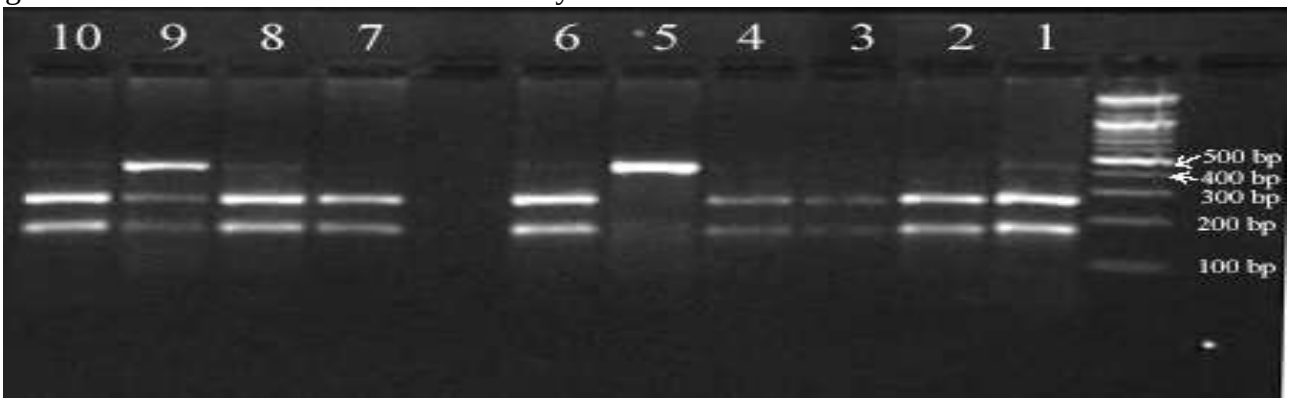

Figure1. shows the digestation of 430 fraqment with *NlaIII* enzyme and the genotypes identified in this study the Lane 1-2-3-4-6-7-8-10 were TT genotype , Lane 9 TC genotype ,Lane 5 was CC genotype

2.Genotype and alleles frequencies

Table 1 shows the genotypes frequencies ocurrding to Lactoferrin gene SNP (+7605 C/T) frequencies of the genotypes determined by chi-square test, The results showed high significant difference in distribution of genotypes at (P<0.01) in the studied population , the TT genotype had the highest frequency 62 followed by the TC genotype 30, The least frequent genotype was CC genotype 8 ,the T allele had higher frequency than C allele 0.77 and 0.23 respectively this due to the high frequency of TT genotype .

Table 1 . Genotypes and allele frequencies of the SNP (7605 C>T) in Lactoferrin gene in Goats

Genotypes	Number	Genotype distribution
TT	31	62
TC	15	30
CC	4	8
Total	50	100%
Chi-square (χ^2)	----	37.160**
Alleles frequency	T	0.77
	C	0.23

** (P<0.01).

3.Association between (+7605 C/T) SNP Genotypes with milk production

The results of the current study showed significant effects (P<0.05) (+7605 C/T) SNP genotypes on daily milk yield individuals with genotype CC had higher mean (0.12±0.950 Lt) than genotype TT (0.08±0.810 Lt) As shown in Table 2, Also significant effects (P<0.05) of genotype on total milk yield individuals with genotype CC had higher mean compared with genotype TT shown in Table 2 .There was no significant difference in lactation period between genotypes . The primary function of lactoferrin lies in its role in iron metabolism including iron transport, storage and chelation. lactoferrin exhibits strong antimicrobial activity against a broad spectrum of bacteria (gram-positive and negative), fungi, yeasts, viruses and parasites. lactoferrin exerts bacteriostatic and bactericidal activity. Its main contribution to antiviral defense consists in its binding to the cell membrane glycosaminoglycan, thus lactoferrin prevents viruses from entering cells and infection is stopped at an early age. More than 140 SNPs in this gene have been identified. Such a high variability in Lactoferrin gene implies that it may be used as candidate gene for screening animals also a marker of milk yield.The effect of Lactoferrin on milk production in all ruminants is similar Lactoferrin secreted in milk protect tissues of mammry gland from infection and pathogenes especially mastitis Consequently some changes and mutations in the lactoferrin gene may lead on positive or negative changes in milk production so the changes in lactoferrin gene can be used as markers-assisted selection .

Table 2. Least square means and standard errors of the milk production in genotypes of the SNP (7605 C>T)

Genotypes	Mean ± SE		
	Daily milk yield (Lt)	Lactation period (days)	Total milk yield (Lt)
CC	0.12±0.950 a	10.57±137.00 a	21.66±130.10 a
TC	0.10±0.853 ab	5.08±135.76 a	10.40±115.28 ab
TT	0.08±0.810 b	4.09±134.35 a	8.39±108.69 b

Means followed by different superscript letters in the same column differ significantly
* (P < 0.05), Non significant :NS

4.Association of (+7605 C/T) SNP genotypes with milk composition

As shown in the table3 there is a significant difference in milk protein (P<0.05) According to the difference genotypes, The CC genotype had higher mean value (0.11±3.35) than TC genotype (0.07±3.07). There was no significant difference between genotypes on the traits :SNF, Fat and Lactose.

Table 3.Least square means and standard errors of the milk composition traits in genotypes of the SNP (7605 C>T)

Genotypes	Mean ± SE(%)			
	Fat	Protein	SNF	Lactose
CC	0.39±2.94 a	0.11±3.35 A	0.40±8.84 a	0.18±4.92 a
TC	0.27±2.47 a	0.07±3.07 B	0.24±8.45 a	0.13±4.72 a
TT	0.20±2.99 a	0.05±3.15 Ab	0.17±8.43 a	0.09±4.32 a
P	NS	*	NS	NS

Means followed by different superscript letters in the same column differ significantly
* (P<0.05), Non significant :NS

The SNP(+7605 C/T) detected in Goats lactoferrin gene has significant effect on milk production and milk protein and this study confirmed the hypothesis that SNP(+7605 C/T) in lactoferrin gene can be used as a marker of milk production and the SNP may be a potential genetic marker in selection programs for dairy goats through marker-assisted selection . Further investigations are needed to confirm these results and determine the mechanisms underlying the effect of lactoferrin gene on milk production traits .

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