ALPHA-LACTALBUMIN GENE POLYMORPHISMS IN RELATION TO MILK PROTEIN CONCENTRATION IN MAGHRABI CAMEL

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Alpha lactalbumin is one of the major proteins of camel milk whey and essential for the biosynthesis of lactose at the level of mammary glands. The objective of this study is identifying genetic polymorphism of the alpha-lactalbumin gene and its relationship with milk protein concentration of Maghrabi camel. Twenty one females of Maghrabi camel belonging to Camel Researche Station, Matrouh, Agricultural Research Center, Ministry of Agriculture and Land Reclamation, Egypt were used. Milk and blood samples were collected for the analysis of milk composition, protein electrophoresis and alpha-lactalbumin gene sequencing. Bioinformatics analysis was done to accomplish the PCR based sequencing technique to investigate the different Single Nucleotide Polymorphisms (SNPs) of alpha-lactalbumin gene. Results of camel milk composition percentages of protein, fat, lactose, total solids and solids not-fat were 3.2, 3.5, 4.8, 12.6 and 9.1, respectively. The alpha-lactalbumin protein band with molecular weight of 14.6 kDa was identified in different density concentrations. Alpha-lactalbumin gene amplified band with about 1000 bp length was detected in all studied samples. After PCR amplification, samples were divided into three groups according to milk protein concentration as follows, high (3.4-3.8%), medium (3.0-3.4%) and low (2.5-3.0%). The obtained sequences were submitted and accepted at the International Gene Bank and got the accession number KF648561.1. Several SNPs were found to be repeated in many camels in the nucleotide position and molecular weights. However, SNPs at positions 255(G-A, 0.279) and 263(C-T, 0.286) of transition types showed apparent association with high milk protein concentrations. The obtained results indicated that the identified SNPs in the alpha-lactalbumin gene affect the milk protein concentration and may be used in camel selection programs.

Keywords: alpha-lactalbumin, protein electrophoresis, genetic polymorphism, Maghrabi camel

Camel produces more milk and for a longer lactation season period than any other dairy farm animal held under the same conditions (Kaufmann and Binder, 2002). Camel milk has greater similarity to human milk and a higher concentration of vitamin C, potassium, and iron; lower concentrations of lactose, β-casein, β-lactoglobulin, cholesterol, and fat when compared to bovine milk (Mullaicharam, 2014 and Uversky et al., 2016).

Alpha-lactalbumin is a major milk protein gene essential for the biosynthesis of lactose at the level of mammary glands. Alpha-lactalbumin directly influences the quantity and the quality of milk (Ashwell et al., 1997). In addition, it regulates cell growth (Sternhagen and Allen, 2001).

Alpha-lactalbumin is one of the major proteins of camel milk whey. Many reports suggest that Single Nucleotide Polymorphisms (SNPs) in alpha-lactalbumin potentially alter the gene expression and may be associated with differences in milk yield and quality (Bleck and Bermal, 1993 and Ramesha et al., 2008). Variation in SNF and contributed proteins may be attributed to polymorphism in sequence of causative genes. Also, Gamez et al. (2012) found two SNPs in Dairy sheep. However, Noce et al. (2016) identified 29 SNPs in Sarda sheep, plus three previously reported SNPs mapped to the alpha-lactalbumin and beta-lactoglobulin genes. Moreover, Rahmatalla et al. (2016) in the Sudanese goat breeds found seventeen SNPs. Whereas, Huang et al. (2012) studied the two major whey protein genes (alpha-lactalbumin and beta-lactoglobulin) that show associations with milk protein in Dairy cattle. Several SNPs were found to significantly associate with milk protein traits for alpha-lactalbumin. These significant SNPs explained a large proportion of the phenotypic variation of milk protein composition and can be used for selecting animals that produce milk with desired composition or desired processing and manufacturing properties. In camels, Saedi et al. (2017) analysed alpha-lactalbumin gene promoter region with about 1020 bp length, which probably is present in both Iranian bactrians and dromedaries camel species. Five haplotypes (4 mutations) in dromedaries and 4 haplotypes (3 mutations) in bactrians were detected.

The objective of the present study was to identify genetic polymorphism of the alpha-lactalbumin gene and its relationship with milk protein concentration trait in Maghrabi camel breed.

MATERIALS AND METHODS

1. Samples Collection

Blood and milk samples were collected from twenty-one Maghrabi camels obtained from Camel Research Station, Matrouh, Agricultural Research Center, Ministry of Agriculture and Land Reclamation, Egypt. Camels were kept under a semi intensive production system and were

housed in open yards. Samples were transported to the laboratory and stored at -20°C for further analysis. Milk composition (protein, fat, lactose, total solids and non-fat solid contents) was determined using a lactoscan milk analyzer (Model Lactoscan SL, Milkotronic Ltd, Bulgaria).

2. SDS-PAGE Analysis

SDS-PAGE was carried out in a vertical gel electrophoresis apparatus (plate size: 160 mm×160 mm) (ATTO Corp., Japan) using 5% (w/v) stacking gel and 12% (w/v) separating gel (Kumar et al., 2013). After electrophoresis, the gels were stained with Coomassie blue R-250 for visualization of the proteins. Molecular weight of the protein bands was determined with reference to standards (SIGMA MARKER, M-4038). Densitometry scanning of the gels was carried out using a Gel Analysis Software, Gene Tool (Mascon Global Ltd.).

3. PCR Amplification and Sequencing

DNA was extracted from the blood samples of 21 Maghrabi camel females using commercially available ReliaPrep™ Blood gDNA Miniprep System kit (Promega Corporation, Madison, USA), according to manufacturer's instructions.

Alpha-lactalbumin primer pairs were designed based on the published nucleotide sequence information of this gene. The primer sequences are shown in table (1).

Table (1). Primer sequences and annealing temperature.

	Primer sequences	**Protocol**
Alpha-lactalbumin	*Up*. 5′- AGTTTGGGGCCAGAGAGAAT-3′ *Down*. 5′-TTTCCCTGTTCAGAGAGCGT-3′	initial denaturation at 94ºC for 4 min followed by 10 cycles (4 min at 94ºC, 1 min at 52ºC, and 1 min at 72ºC), then 15 cycles (4 min at 94ºC, 1 min at 58ºC, and 1 min at 72º C).

PCR reactions were carried out in a total volume of 25 µl containing 3 µl of genomic DNA, 12.5 µl of GO Taq Green Master Mix, 1 µl of forward primer, 1 µl of reverse primer and 7.5 µl of nuclease-free water. The amplified DNA fragments were separated on 1.5% agarose gel (Bioshop Canada, Burlington, Ontario, Canada), stained with ethidium bromide (Bioshop Canada), visualized on a UV Trans illuminator, photographed by Gel Doc. BIORAD 2000 and analysed with software data analysis for Bio-Rad Model 620 USA. The specific band was cut from the gel and purified by

gel purification kit (Jena Bioscience) according to the manufacturer's instructions. Sequencing was carried out by Ready Reaction Kit (ABI Applied Biosystems, Foster City, California, USA) on a 3130XL Genetic Analyzer (Applied Biosystems). The obtained sequences were analysed using Basic Local Aligned Tool (BLAST) online on the National Center for Biotechnology Information (NCBI) to determine the Single Nucleotide Polymorphism (SNP). Multi sequence alignments were analyzed using BIO EDIT V3 program. Pearson correlation between milk protein concentration and SNPs were calculated and tested for significantly according to SPSS (2012).

RESULTS AND DISCUSSION

1. Milk Composition

Camel milk composition showed averages of 3.2, 3.5, 4.8, 12.6 and 9.1% for protein, fat, lactose, total solids and solids not-fat (SNF), respectively. But, protein % was ranged from 2.5 to 3.8% among individual camels. These results are in agreement with Ibrahim (2016), who found that concentration values of protein, fat, lactose and total solids in Maghrabi camel's milk were 3.25, 3.2, 4.5 and 12.15%, respectively. According to most authors, the composition of camel milk varies accordance to differences of geographical origin and year of publication of the published data, beside other factors such as the physiological stage, feeding conditions, seasonal or physiological variations, genetic or health status of camel, which have also a paramount importance (Konuspayeva et al., 2009).

2. Milk Protein Electrophoresis

The protein fractions of camel were analyzed by SDS-PAGE (Fig. 1, 2) and identified on the basis of their molecular weight in comparison with the marker protein ladder. Most of the milk samples presented a common band of 14.6 kDa molecular weight but with different intensities. This band corresponds to alpha-lactalbumin, which is more intense in camel milk with 72% of homogeneity. This observed band with different density concentrations might due to individual variation in milk protein concentration. This result is in full agreement with that obtained by Obaid Ullah et al. (1985), who showed that camel alpha-lactalbumin has 123 residues and a molecular mass of 14.6 kDa. Also, Kappeler (1998) reported that camel alpha-lactalbumin consists of a 123 amino acid with a molecular weight of 14.42 KDa. This slight difference in camel alpha-lactalbumin molecular weight may be due to genetic differences between camel breeds.

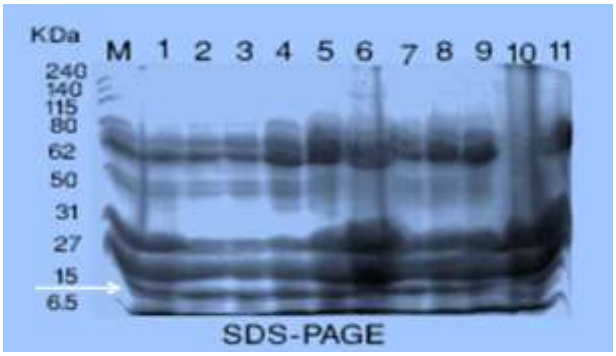

Fig. (1). Milk protein electrophoresis of Maghrabi camel (1-11).

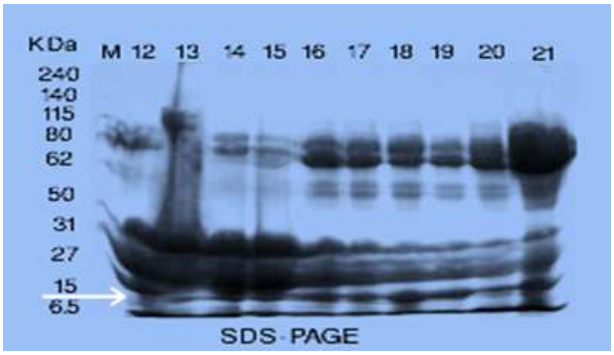

Fig. (2). Milk protein electrophoresis of Maghrabi camel (12-21).

3. Molecular Genetic Analysis

In the present study, bioinformatics tools were utilized to synthesize specific PCR primers for amplifying alpha-lactalbumin gene. The specificity of synthesized primers was examined in silico. Alpha-lactalbumin gene amplified band with about 1000 bp length was detected in almost all studied samples of Maghrabi camel (Fig. 3 and 4). This band size was similar to that reported by Saedi et al. (2017) when they studied of the same region in Iranian bactrians and dromedaries camel species. The samples were divided into three groups according to milk protein concentration as high (3.4-3.8%), medium (3.0-3.4%) and low (2.5-3.0%).

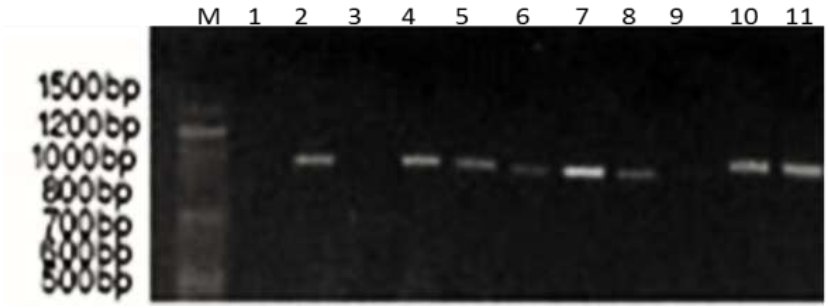

Fig. (3). Electrophoresis pattern of amplified Maghrabi camel genomic DNA with alpha-lactalbumin specific primer separated in 2% agarose gel**.** Molecular size marker (50 bp DNA Step Ladder). Lanes 1-11: fragments amplified.

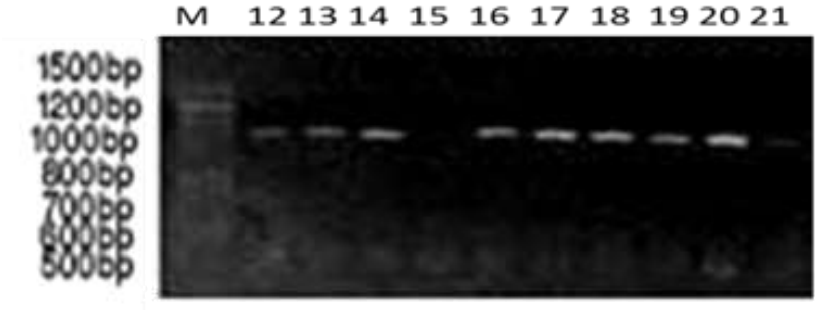

Fig. (4). Electrophoresis pattern of amplified Maghrabi camel genomic DNA with alpha-lactalbumin specific primer separated in 2% agarose gel**.** Molecular size marker (50 bp DNA Step Ladder). Lanes 12-21: fragments amplified.

The amplified PCR band was purified and sequenced, then the obtained sequence was alignment for the alpha-lactalbumin gene in Maghrabi camels with the available sequence at the Gene Bank, and the observed nucleotide change are shown in fig. (5) and table (2). The number of recorded SNPs ranged from 14-35 with sequence identity ranging from 77-90%. The obtained sequences were submitted and accepted at the International Gene Bank and got the accession number of KF648561.1.

Eleven SNPs were found to be repeated in many camels in the nucleotide position and molecular weights of 115, 116, 117, 125, 129, 152, 204, 220, 252, 255 and 263. But the SNPs at positions 255(G-A, 0.279) and 263(C-T, 0.286) transition types are highly significantly ($p< 0.01$), linked with high milk protein concentration with correlation coefficient of 0.435. The presence of these SNPs explains the differences between the Maghrabi camels in protein concentration.

Similar results were obtained by Saedi et al. (2017), who found alpha-lactalbumin gene promoter region with about 1020 bp length in both Iranian bactrians and dromedaries camel species. They reported five haplotypes (4 mutations) in dromedaries and 4 haplotypes (3 mutations) in bactrians. Also, several previous studies identified SNPs in alpha-lactalbumin for many species (Bleck and Bremel, 1993), two allele's polymorphism among alpha-lactalbumin in Holstein cattle were also identified. Cows with allele A of the gene have higher milk yield, protein yield, and fat yield; the B allele is associated with a higher percentage of protein and fat. Ramesha (2006) screened six indigenous Cattle breeds of Southern India for SNPs in the coding sequence of the bovine alpha-lactalbumin gene. SNPs at four positions (772, 775, 792 and 857) in exon 1 and three positions at 1231, 1264 and 1335 in exon 2 were observed. They further interpreted the effect of these SNPs in the mature protein based on the structure of bovine alpha-lactalbumin. In addition, Huang et al. (2012) studied the 2 major whey protein genes (alpha-lactalbumin and beta-lactoglobulin) in dairy cattle. They found several SNPs, which were significantly associated with milk protein traits. These significant SNPs explained a large proportion of the phenotypic variation of milk protein composition and can be used for selecting animals that produce milk with desired composition or desired processing and manufacturing properties**.** However, Gamez et al. (2012) found two alpha-lactalbumin SNPs in Dairy sheep. Noce et al. (2016) also identified 29 SNPs in Sarda sheep, in addition to three previously reported SNPs mapping for the alpha-lactalbumin and beta-lactoglobulin genes. While, Rahmatalla et al. (2016) found seventeen alpha-lactalbumin SNPs in the Sudanese goat breeds.

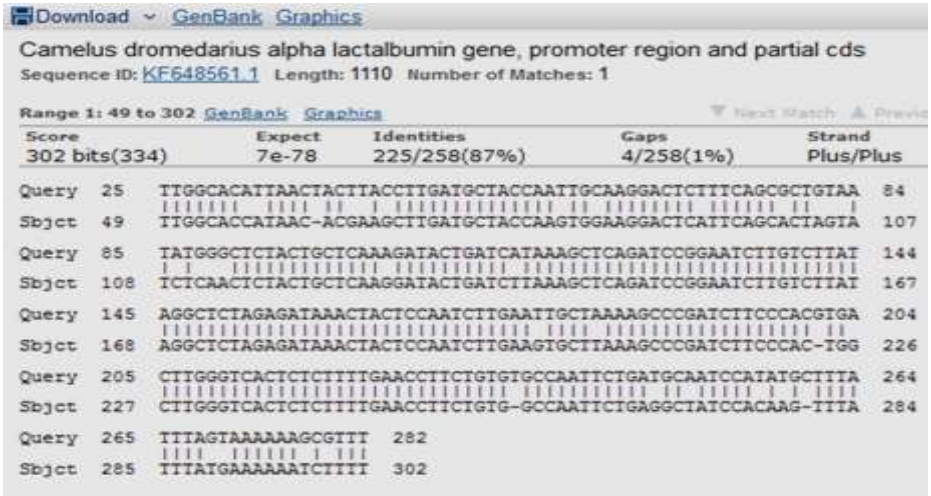

Fig. (5). Sequence alignment for the alpha-lactalbumin gene in Maghrabi camels with available sequence at the Gene Bank of KF648561.1.

Table (2). Summary of the observed nucleotide change for alpha-lactalbumin gene in promoter region of Maghrabi camels.

Animal	No. of change	Identity %	Nucleotide change position	Protein %
1	19	90	33, 42, 43, 45, 55, 60, 63, 68, 116, 117, 124, 125, 129, 130, 139, 153, 155, 162 and 167	2.5
2	14	90	72, 78, 81, 82, 83, 86, 88, 89, 90, 104, 115, 176, 182 and 204	2.6
3	18	90	115, 116, 117, 124, 125, 129, 130, 139, 153, 155, 162, 167, 168, 170, 173, 175, 179, 185 and 203	2.8
4	24	87	33, 63, 72, 78, 81, 82, 83, 86, 88, 89, 90, 104, 115, 176, 182, 204, 220, 245, 248, 252, 270, 277 and 279	3.0
5	20	87	78, 81, 82, 83, 86, 89, 90, 104, 115, 176, 182, 204, 220, 245, 248, 252, 270, 277 and 279	3.2
6	35	77	104, 112, 115, 116, 117, 124, 125, 129, 130, 139, 153, 155, 162, 167, 168, 170, 173, 175, 179, 185, 186, 200, 203, 204, 208, 210, 211, 218, 220, 242, 244, 246, 252, 255 and 263	3.4
7	29	77	115, 125, 129, 130, 139, 153, 155, 162, 168, 170, 173, 175, 179, 185, 186, 200, 203, 204, 208, 210, 211, 218, 220, 242, 244, 246, 252, 255 and 263	3.6
8	31	77	109, 112, 115, 116, 117, 124, 125, 129, 130, 139, 153, 155, 162, 167, 168, 170, 173, 175, 179, 185, 186, 200, 203, 204, 220, 242, 244, 246, 252, 255 and 263	3.8

The obtained results indicated that the identified SNPs in the alpha-lactalbumin gene promoter region may have an effect on the milk protein and lactose concentrations. The current findings could be used for selecting camels that produce much more milk with higher protein concentrations and more lactose.

CONCLUSION

To the best of our knowledge, this is a pioneer study involving association between alpha-lactalbumin gene and milk protein concentration in Maghrabi camel breed. The SNPs explained a phenotypic variation of milk protein concentration, which may be used for selecting camels that produce more total milk yield with higher protein concentrations, as well as more lactose synthesis. Further functional analysis and genetic association studies using larger samples are probably needed to ensure the exact role of this gene in the camel breeds.

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تعدد الأشكال المظهرية لجين الفالاكتالبومين وعلاقتها بتركيز بروتين اللبن في الإبل المغربي

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ألفالاكتالبومين هو أحد البروتينات الرئيسية في شرش ألبان الإبل وهو أساسي للتخليق الحيوي للاكتوز على مستوى الغدد الثديية، لذلك كان الهدف من هذه الدراسة هو التعرف على تعدد الأشكال المظهرية لجين ألفالاكتالبومين وعلاقته بتركيز البروتين في لبن الإبل المغربي. وقد تم إستخدام واحد وعشرون من إناث الإبل المغربي، والمرباة في محطة بحوث الإبل بمطروح التابعة لمركز البحوث الزراعية، وزارة الزراعة وإستصلاح الأراضي، مصر. تم تجميع عينات الدم والحليب لتحليل مكونات اللبن والتفريد الكهربي للبروتينات ودراسة تسلسل جين ألفالاكتالبومين. وقد تم إستخدام Bioinformatics analysis لدراسة SNPs المختلفة في تسلسل جين الألفا لاكتالبومين. وقد أظهرت النتائج أن النسب المئوية لمكونات لبن الإبل من البروتين والدهون واللاكتوز والمواد الصلبة الكلية والمواد الصلبة غير الدهنية كانت 3.2، 3.5، 4.8، 12.6 و9.1٪، على التوالي. وأوضحت نتائج التفريد الكهربي للبروتين أن الوزن الجزيئي للألفالاكتالبومين كان 14.6 كيلو دالتون مع وجود إختلاف في تركيزالكثافة حيث ظهرت حزم جين ألفالاكتالبومين في جميع العينات المدروسة وكان طول الجزء الذي تم دراسته حوالي 1000 bp. تم تقسيم العينات إلى ثلاث مجموعات وفقًا لتركيز بروتين الحليب على النحو التالي: المرتفعة (3.4-3.8٪)، المتوسطة (3.0-3.4٪) والمنخفضة (2.5-3.0٪). تم مطابقة تسلسل جين ألفالاكتالبومين المتحصل عليه في بنك الجينات الدولي .KF648561.1، حيث تم تحديد العديد من SNPs بصورة متكررة في الكثير من الإبل في نفس موقع النوكليوتيدات والوزن الجزيئي، ومع ذلك كان هناك 2 SNPS وهما 255(G-A, 0.279) و263(C-T, 0.286) transition types مرتبطة بزيادة تركيزالبروتين. وقد خلصت الدراسة إلى أن SNPs في جين الفالاكتالبومين التي تم تحديدها كان لها تأثير على تركيز بروتين اللبن ويمكن إستخدامها في برامج إنتخاب وتحسين إنتاجية الإبل.