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ROLE OF GENETIC VARIATION OF αS1-CASEIN, LACTATION TIME AND PARITY ON MILK PRODUCTION AND COMPOSITION OF ZARAIBI GOATS

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ABSTRACT

The aim of this study was to investigate the effect of CSN1S1 gene polymorphism, time of lactation and parity on milk quantity and quality. A total number of 165 Zaraibi goats (105 kids, 7 bucks and 53 does) after kidding season of February/ March 2013 were used in this experiment. The electrophoretic pattern of digested fragments of PCR amplified goat CSN1S1 products with ***XmnI*** enzyme showed the presence of 9 genotypes, 4 homozygous (AA, BB, CC and DD) and the other 5 heterozygous (AC, AD, BC, BD and CD). The nine genotypes were derived from 4 alleles; three strong alleles (A, B and C) with frequencies (17.76, 27.63 and 26.97%, respectively) and one intermediate allele (D) with frequency 27.63%. BB and CC were the most common homozygous genotypes detected in the present study with similar frequency (18.42%) followed by DD (17.76%), BD (13.82%), AA (10.53%), AC (10.53%), BC (4.61%), AD (3.95%) and CD (1.97%). Daily milk yield, percentage of milk fat, protein, total solid and solid not fat significantly affected by CSN1S1 genotypes. Zaraibi does carrying AD variant showed the highest levels of daily milk yield, fat, protein, total solid and solid not fat. Sequence analysis showed that only the deletion of nucleotide C in allele A caused substitution of TTC to TTG forming Leu (Leucine) instead of Phe (Phenyl alanine) in mature protein. Meanwhile, no polymorphism detected between nucleotide 1 and 274 at amplified sequence of exon 9, so this region is considered as a highly conserved among alleles (A, B, C and D).

Daily milk yields in Zaraibi does were significantly high at the first week after kidding, peaked at the 2nd week then decrease till the end of lactation. Moreover, the highest milk yields were recorded in does at the 7th and 6th parities, while the lowest values observed in does at the 1st and 2nd parities. In addition, percentages of fat and total solids were the highest at day 90 (end of suckling period) than during days 120 and 210 of lactation period. Milk protein was the highest at end of lactation (day 210), while lactose levels showed the lowest values.

In conclusion, studying genetic polymorphism for Zaraibi goats CSN1S1 gene is important for identifying favorable genotypes associated with high milk yields, protein and fat. Breeding program would be changed toward selecting animals carrying the strong and mild alleles for milk protein and fat required for economic goat milk industry.

Key words: Goats, CSN1S1 polymorphism, lactation time, parity, milk yield, milk composition.

INTRODUCTION

In Egypt, Zaraibi (Egyptian Nubian) goats are the most promising dairy goat among the local Egyptian breeds. They characterize by high genetic potential as a dairy and prolific breed which reared mainly for milk production **(Marai *et al*., 2001 and Galal *et al*., 2005)**. Interest in the use of goat milk has been increased lately, as goat's milk composition is very similar to cow's milk which make it widely used for processing (consume goat milk, cheese, yoghurts, fermented milk products, etc.). Another important aspect to be considered is the growing importance of the use of goat milk as an alternative to cow milk in the infant diet because of its digestibility and low allergicity **(Mowlem, 2005)**.

Ruminant milk production and composition is influenced by several factors as breed, age, stage of lactation, nutrition, environment and genetic factors **(Clark and Sherbon, 2000)**.

In goat milk, the main protein fractions are caseins. They affect the physiochemical, nutritional and technological properties of milk and thus exhibit both quantitative and qualitative variation arising from genetic polymorphism in the encoded genes **(Ramunno *et al.*, 2000 & 2001)**. They proposed as polymorphic markers for the selection in order to improve yield and quality of milk **(Othman *et al.*, 2013)**. Four caseins were expressed in goats' milk (αs1, αs2, β- and κ-caseins) coded by the CSN1S1, CSN1S2, CSN2 and CSN3, respectively. They are located within 250-kb segment of caprine chromosome 6 **(Martin *et al*., 2002)**. CSN1S1 is the most polymorphic casein gene, where more than 17 alleles (A, B1, B2, B3, B4, C, D, E, F, G, H, I, L, M, N, 01 and 02) have been detected and grouped into 4 classes according to its expression levels in milk. Each allele has unique characteristic from single nucleotide substitution/ deletions to large insertion/ deletions or interallelic recombination **(Cosenza *et al*., 2003 and Ramunno *et al*., 2005)**.

Since, milk quantity and quality varies greatly between different genotypes raised at different locations, detection of candidate genes and suitable genetic markers for milk production traits are very important. So, this study was designed to investigate the effect of genetic polymorphism of CSN1S1 gene, as well as, lactation time and animal parity on milk yield and composition in Zaraibi goats.

MATERIALS AND METHODS

Animals and Management

This study was carried out at Sakha Experimental Station, Kafr El-Sheikh, Animal Production Research Institute (APRI), Agriculture Research Center (ARC), Ministry of Agriculture and Land Reclamation (MALR).

A total number of 165 Zaraibi goats (105 kids, 7 bucks and 53 does) were chosen after kidding season of February/ March 2013. Does weight ranged from 28 to 42 kg and age between 24 to 108 months. All animals were fed according to **NRC (2007)** allowances. Water and salt mineral blocks were available all the time.

Milk and blood samples

Milk yields of 50 Zaraibi does were recorded during suckling at days 7, 14, 30, 60 and 90 and during lactation period at days 120 and 210 for daily and total milk yield calculation. During suckling, milking was done using oxytocin technique according to **Banda *et al*. (2006)**. Meanwhile, does were machine milked during lactation period. The amount of milk measured on day 7 after kidding multiplied by 7 to calculate the yield of the first week. Average milk yields at days 14, 30, 60, 90, 120 and 210 were calculated by multiplying daily milk records by the number of days. Moreover, total milk yield was estimated by summing milk yields taken during suckling and lactation periods.

Ten ml of blood samples were collected from all animals on ethylenediaminetetraacetic acid (EDTA) as anticoagulant and kept in -20°C for DNA extraction.

DNA extraction, Quantification and Sequencing

Genomic DNA was extracted from all collected blood samples using salting out method as described by **Miller *et al.* (1988)**. The concentrations and purity of extracted DNA were measured using spectrophotometer (Eppendorf Biophotometer plus) at wavelengths 260 and 280 nm.

PCR Amplification was performed on Bio Rad thermal cycler (model C1000). Amplification protocol of CSN1S1was carried out according to **Ramunno *et al.* (2000)** with changing annealing temperature to 59ºC (Table 1). Moreover, restriction analysis was performed by using Fast Digest ***XmnI*** restriction enzyme (Cat. No.: #FD1534).

Allele specific PCR (AS-PCR) method was used to distinguish allele A from 0 **(Cosenza *et al.*, 2003)** and allele B from E **(Feligini *et al.*, 2005)**. The primers used for AS- PCR are illustrated in Table (1).

PCR and PCR- RFLP products were run directly by electrophoresis using 1% agarose gel that stained with ethidium bromide in 1X TBE (Tris- base buffer), where GeneRuler 50 bp (Thermo Scientific) was used as molecular size marker. The bands were visualized under ultra-violet (UV) light and photographed with Gel Documentation system (SynGene, GeneTools-File version: 4.02.03, France). Gel-Pro Analyzer program (Media Cybernetics, Silver Spring, MD, USA, v3.1.0.0) was used to estimate the size and quantity of the band representing each allele.

In order to determine genetic polymorphism of CSN1S1 in Zaraibi goats, the region between nucleotides 208 and 420 (part of intron 8, exon 9 and part of the intron 9) was amplified (Accession no. X56462.1). Cycle sequencing reaction was performed using BigDye® Terminator (v3.1) cycle sequencing Kit (Applied Biosystems). Amplicons representing unique banding patterns of CSN1S1 gene was sequenced using automated DNA ABI Prism (3130) Genetic Analyzer based on dideoxynucleotide chain-termination technique **(Sanger *et al.*, 1977)**. Multiple Sequence alignment was performed with BioEdit sequence alignment editor program (v7.2.5). The BLAST algorithm was used to search the NCBI (National Center for Biotechnology Information) Gen Bank **(http://www.ncbi.nlm.nih.gov/)** for homologous sequences of CSN1S1 in goats.

Milk Analysis

Milk samples from morning milking at days 90, 120 and 210 were used for chemical analysis. Milk components (percentage of fat, protein, lactose, total solid and solid not fat) were estimated using infra-red spectroscopy (Milko-Scan 133B; N. Foss Electric, DK 3400 Hillerod, Denmark) according to **(Smith *et al.*, 1993 and Lynch *et al.*, 2006)**.

Statistical Analysis

Genotypes were estimated by counting the electrophoretic pattern of PCR amplified goat CSN1S1 products. Alleles and genotypes frequencies were calculated according to the formulas:

$f(A) = [(AA*2+AC+AD)/2N]$ for alleles frequencies.

$f(AA) = f(A)*f(A)*N$, for homozygous genotype frequencies

$f(AC) = f(A)*f(C)*N$ for heterozygous genotypes frequencies,

where A is allele and AA, AC and AD were the counted genotype and N is number of tested individuals.

The calculated frequencies for alleles and genotypes should verify Hardy-Weinberg equilibrium where $[f(A)+f(B)+f(C)+f(D)]^2=1$

All collected data were statistically analyzed using General Linear Models **(SAS, 2000)**. Results were expressed as least square means (LSM± SE). Two models were used in this study, the first model was to estimate the effect of time and parity on daily milk yields, while the second one was used to explore the effect of CSN1S1 genotypes on daily milk yield and composition.

First model:

$Y_{ijk}= \mu+T_i+P_j+e_{ijk}$

Where,

μ = the overall mean,

Y= the observed records of daily milk yield and milk composition,

T_i= the fixed effect of i^{th} time of daily milk yield (i=7, 14, 30, 60, 90, 120, 210) or the fixed effect of time of milk composition (i = 90, 120, 210),

P_j= the fixed effect of j^{th} parity of does (j=1,……7),

e_{ijk}= Random error.

Second model:

$Y_{ij}=\mu+GA_i+e_{ij}$

Where,

μ = the overall mean,

Y= the observed records of daily milk yield and milk composition (at 90, 120, 210 days).

GA_i= the fixed effect of i^{th} CSN1S1 genotypes (i = AA, BB, CC, DD, AC, AD, BD),

e_{ij}= Random error.

RESULTS AND DISCUSSION

CSN1S1 Polymorphism

Figure 1 showed the electrophoretic pattern of PCR amplified goat CSN1S1 products at 223 bp. After restricted digestion with ***XmnI*** enzyme**,** nine genotypes derived from four alleles (A, B, C and D) were observed. Four genotypes were homozygous (AA, BB, CC and DD) and the other five were heterozygous (AC, AD, BC, BD and CD). The fragments of AA, BB, CC and DD genotypes were detected at 150, 161, 212 and 223 bp, respectively (Fig. 2 a). The heterozygous genotypes were characterized by the presence of two fragments (Fig. 2 b): AC (at 150 and 212 bp), AD (at 150 and 223 bp), BC (at 161 and 212 bp), BD (at 161 and 223 bp) and CD (at 212 and 223 bp). Results of AS-PCR products showed the presence of fragment characterizing the A allele at 700 bp, where no 0 allele (216 bp) was observed (Fig. 3a). Only one fragment at 90 bp was detected indicating the presence of B allele. Meanwhile, the fragment specific for E allele (at 550 bp) was not observed (Fig. 3b).

Frequencies of alleles and genotypes estimated in 152 Zaraibi goats out of 165 are illustrated in Table (2).

Rando *et al*. (2000) and Ramunno *et al*. (2005) reported that CSN1S1 has 16 alleles grouped into four groups according to rates of αs1-casein synthesis, where A, B1, B2, B3, B4, C, H and L were strong alleles (produce 3.5 g/l), E and D (intermediate alleles, produce 1.1 g/l), F and G (weak alleles, produce 0.45 g/l) and null alleles (01, 02 and N, produce no protein). Three strong alleles (A, B and C) with frequencies (17.76, 27.63 and 26.97%, respectively) and one intermediate allele (D) with frequency 27.63% were detected in the present study. Moreover, the most common genotypes observed were BB and CC with similar frequency (18.42%) followed by DD (17.76%), BD (13.82%), AA (10.53%), AC (10.53%), BC (4.61%), AD (3.95%) and CD (1.97%). **Ahmed (2006) and Ahmed and Othman (2009)** observed three genotypes in Zaraibi goat (BD, BB and AC) with different frequencies than that reported in our study (80, 10 and 10%, respectively). In other goat breeds, **Sztankoova *et al*. (2006)** found AA genotype with frequencies 0.95 and 0.98 in dairy White and Brown short haired goats, respectively. **Vacca *et al*. (2009)** recorded frequencies 26.6 and 34.2% for A and B alleles, respectively and 5.41 and 7.21% for AA and BB genotypes, respectively in Tunisian native goats. Moreover, **Rout *et al.* (2010)** observed CSN1S1*A with frequency 77.4 and 45% in Barbari and Ganjam Indian goats, respectively. They also detected CSN1S1*B with very low frequency in Barbari goats (1.1%). **Pazzola *et al.* (2014)** recorded the frequency of genotypes AA and BB as 7 and 27.5%, respectively in Sarda goat breed.

Effect of CSN1S1 Genotypes on Milk Yield and Composition

LSM values of milk yield and composition as affected by CSN1S1 genotypes are illustrated in Table (3).

Daily milk yield was significantly ($p<0.05$) affected by CSN1S1 genotypes (Table 3). Does with AD variant have the highest daily milk yield (2.00±0.18 kg), while does carry DD genotype showed the lowest level (1.07±0.09 kg). **Pazzola *et al.* (2014)** reported significant effect of CSN1S1 locus on daily milk yield. They estimated the highest milk yield in Sarda does with AA genotype, while the lowest value observed in does carry FF genotype. Meanwhile, **Balia *et al*. (2013)** found that AA and BB genotypes did not significantly affect daily milk yield in Sarda goat.

Percentages of milk fat, protein, total solid and solid not fat were significantly ($p<0.05$) affected by CSN1S1 genotypes (table 3). Does carrying AD genotype showed the highest fat, protein, total solid and solid not fat levels (3.96±0.47, 3.84±0.35, 12.30± 1.11 and 8.71±0.51 %, respectively) comparing to those with other genotypes (Table 3). Meanwhile, does carrying AA genotype showed the lowest fat, protein, total solid and solid not fat levels (2.95±0.22, 2.55±0.17, and 9.65±0.52 and 7.16±0.24 %, respectively). Although, no significant differences were detected in lactose level in Zaraibi goats, does carry BB and AD genotypes showed the highest lactose percent (4.17±0.16 and 4.16±0.42 %, respectively). **Clark and Sherbon (2000)** observed that average values for all milk components were the lowest in milk with homozygous genotypes and the highest in samples containing high type of genetic variant in combination with any other type of variant. They also detected that samples containing at least one high type variant tended to have higher coagulation rate, curd firmness and coagulation time. In Cilentana goats, **Zullo *et al*. (2005)** recorded significant effect of CSN1S1 genotypes on milk components, where the highest percentages of fat, protein and total solid were recorded in does carry AB genotype and the lowest value in does with FF genotype. They also observed the highest and lowest lactose percentage in does with FF and AB, respectively. **Pazzola *et al.* (2014)** reported that CSN1S1 locus significantly affect protein levels in Sarda goat, where the highest levels were recorded in goats with AB (4.19%) and the lowest value in those carry AF genotype (3.78%). Meanwhile, **Balia *et al*. (2013)** reported that the CSN1S1 genotype had no significant effect on fat and lactose content but it affected significantly protein levels in Sarda goat. They observed the highest protein level in does with BB genotype, while the lowest level recorded in does carrying AF and BF genotypes.

Sequence Analysis

Alignment of the tested alleles (A, B, C and D) with *Capra hircus* CSN1S1 sequence from Gene bank is shown in Figure (4).

Results showed no polymorphism in the region between nucleotide 1 and 274 at amplified sequence of exon 9, so this region consider as a highly conserved among alleles (A, B, C and D). A deletion of A, T and TG at positions 347, 378 and 362, respectively, as well as, a transition of T-C in position 382 in allele B were detected. Meanwhile, allele D showed a deletion of G (370), T (358), and GT (344), as well as, a transversion of C-A and T-A in positions 386 and 387, respectively. A deletion of T (389) and C (275) were detected in allele A, while both allele A and D showed a deletion of T in position 362. Moreover, alleles B and D have a deletion of T at the two positions (327 and 333), while an insertion of T in position 384 was observed in alleles C and D. Results showed that only the deletion of C in allele A caused substitution of TT<u>C</u> to TT<u>G</u> forming Leu (Leucine) instead of Phe (Phenyl alanine) in mature protein. Meanwhile, the others mutations did not result in any change in amino acid chain (silent mutation). **Caroli *et al*. (2006)** observed silent mutation in CSN1S1*B allele, where a synonymous transversion TC<u>G</u>→TC<u>T</u> (Ser_{66}) occurred in the mature protein. In addition**,** **Ramunno *et al*. (2005)** recorded mutations in allele F, which differentiate it from the other alleles. They also estimated the presence of short insertions of 11 bp (CGTAATGTTTC) between nucleotides (9972–9982) and 3 bp (AAT) between nucleotides (10639–10642) inside the 9th intron. Moreover, they recorded a deletion of 7 bp (TTATCTA) at the 14th intron (nucleotides 14647-14648).

Effect of Lactation Time and Parity on Milk Yield

Least square mean (LSM) levels of daily and total milk yields in Zaraibi does during suckling (days 7, 14, 30, 60 and 90) and lactation (days 120 and 210) as affected by lactation time and parity are represented in Table (4).

Results showed that the overall mean value of total milk yield in Zaraibi goats during 210 days was 317.69± 19.59 kg. In Zaraibi does, **El-Saied *et al.* (2007)** and **Habeeb *et al*. (2009)** reported lower milk yields than that recorded in the present study (249 and 191.46 kg, respectively) during lactation period (210 and 180 days, respectively). Meanwhile, **Abdelhamid *et al*. (2012)** estimated a total milk yield of 363.15 kg during 240 days.

Daily and total milk yield was significantly affected by lactation time and parity ($p<0.0001$). Daily milk yields differ significantly during suckling and lactation period; being high at the first week (2.65±0.24 kg) peaked at the 2nd week (2.73±0.24 kg) then decreased gradually until the end of lactation (Fig. 5). In general, milk yield tended to decrease throughout suckling and lactation periods. Similar result was reported by **Abdelhamid *et al*. (2012)** in Zaraibi goats**.** Meanwhile**, Hamed (2010)** recorded a peak of milk yield at the 4th week of lactation (1.80±0.02 kg) in Zaraibi goats. In other goat breeds, **Strzałkowska *et al*. (2010)** and **Kralickova *et al*. (2013)** observed that daily milk yield decreased significantly from the beginning until the end of lactation in Polish White Improved and Brown short-haired goat. In contrast, **Vacca *et al.* (2010)** and **Mestawet *et al*. (2012)** estimated a peak of daily milk yield at the mid of lactation, while the lower values detected in the early and late lactation stages in Sarda and Ethiopian goats. The differences between levels of daily and total milk yield detected in this study and other studies on Zaraibi goats may be due to individual variations, climate change, feeding regime and method used for milking.

Results in Table (4) and Figure (5) showed that does with higher parities had significantly higher daily milk yield, where the highest milk yields were recorded in does with the 7th and 6th parities (2.00±0.22 and 2.06±0.17 kg, respectively). Meanwhile, the lowest values were observed in does with the 1st and 2nd parities (1.22±0.09 and 1.39±0.07 kg, respectively). In general milk yield increased with the increase of parity number (Fig. 6). In Zaraibi goats, **Teleb *et al*. (2009)** reported that daily milk yield was significantly affected by parity number, where the highest milk yield observed in goats at the 3rd and the 4th parity. **Hamed (2010)** recorded the lowest milk yield in Zaraibi does at the 1st parity. **Anwar *et al.* (2012**) found that milk yield increased with the advancement of parity number, where the highest values recorded at the 3rd, 4th and 5th parities in Anglo-Nubian, Angora, Baladi, and Damascus goat breeds. **Kralickova *et al.* (2013)** reported that brown short-haired goat at the 1st parity showed the lowest daily milk yield, while the highest levels were recorded in goats at the 2nd and 3rd parity. In contrast, **Strzałkowska *et al.* (2010)** estimated the highest daily milk yield in goats at the 1st parity.

Effect of Lactation Time and Parity on Milk Composition

Milk compositions estimated in milk samples of Zaraibi does during lactation period at days 90, 120 and 210 as affected by lactation time and parities are shown in table 5 and figure 6.

The overall mean percentage of fat, protein, lactose, total solid and solid not fat recorded in the present study were 3.31, 2.68, 4.12, 10.73 and 7.5%, respectively. **Teleb *et al.* (2009)** estimated lower levels of fat, protein, lactose, total solid and solid not fat percents (2.9%, 2.5%, 3.8%, 9.8% and 6.9%, respectively) in healthy Zaraibi goats. Meanwhile, **Fernandez *et al*. (2008)** recorded fat, protein, lactose and total solid percent to be 3.7, 2.7, 4.5 and 11.9%, respectively in Mexico goats.

Percentage of fat, protein, lactose and total solid were significantly (p<0.0001, 0.0001, 0.002 and 0.0003, respectively) affected by lactation time. Meanwhile, no significant changes in percentage of solid not fat were recorded. Figure 7 showed that mean values of fat and total solid were the highest at day 90 of lactation (3.91±0.16 and 11.21±0.39 %, respectively) comparing to days 210 (3.60±0.18 and 10.82±0.42 %, respectively) and days 120 (2.56±0.17 and 9.58±0.41 %, respectively). Moreover, milk protein showed the highest level at the end of lactation (day 210, 3.28±0.13 %), while lactose values were the lowest (3.66±0.16 %). Similar results were recorded for fat, total solids and protein percent by **Abdelhamid *et al*. (2012)** in Zaraibi goats, except for lactose levels where the highest values recorded at days 90 and 210. **Strzałkowska *et al*. (2010)** reported high fat and protein levels in late lactation than in early and mid lactation, while lactose levels decreased during lactation process. They also reported, a significant higher total solid in the early and late lactation stage than in the mid lactation in Polish White Improved goats, which agree with our result. Moreover, **Mestawet *et al*. (2012)** reported that fat, protein and total solid in milk were significantly higher in the early and late lactation than that in mid lactation in different goat breeds in Ethiopia.

In the present study, does parity had no significant effect on milk composition (Table 5). However, does with the 6th parity showed the highest fat, total solid and solid not fat percent, while lactose and protein values were the highest in does with the 2nd and 7th parity. **Fernandez *et al*. (2008)** reported that the highest level of fat and total solid was at 7th parity, while protein and lactose showed the highest level at 2nd and 1st parity, respectively in Mexico goats. **Teleb *et al*. (2009)** reported significant effect of parity number on milk protein, total solid and solid not fat% in Zaraibi goats, where the highest levels observed at the 6th parity. They also reported, significantly high fat% in does with the 1st parity. **Addass *et al*. (2013)** observed that parity had no significant effect on protein and total solid levels, but it significantly affect fat and lactose levels showing the highest value (5.54 and 5.30%, respectively) at the 3rd parity in Nigerian goat breed. Meanwhile, **Kralickova *et al. (*2013)** found that the brown short-haired goats on the 2nd parity had significantly higher milk total solid and fat than those on the 1st and 3rd parity, while lactose content show the highest value in goats with 1st parity comparing with 2nd and 3rd parity.

CONCLUSION

In the present study, we confirm the effect of CSN1S1 locus polymorphism, lactation time and parity on milk yield and composition in Zaraibi goats. Two strong homozygous genotypes (BB and AA) with frequency (18.42 and 10.53%, respectively) and one intermediate genotype (DD, 17.76%) were detected in Zaraibi goats. The presence of AA and BB genotypes with high frequency found to be associated with high content of αS1casein used for cheese industry **(Ahmed, 2006)**, where goats carrying those genotypes produced milk characterized by a minor diameter of micelles, significantly higher percent of protein, fat, total calcium and better parameter for crud firming time, crud firming and cheese yield **(Martin *et al*., 1999)**. Meanwhile, the presence of DD genotype with high frequency is favorable for nutritional purpose (as allergic) especially infant diet, as goat milk with low casein is less allergenic than cow's milk (**Roncada, *et al.* (2002) and Ahmed and Othman (2009).** In addition, Zaraibi does with AD variant showed the highest daily milk yield, fat, protein, total solid and solid not fat levels, so it is recommended to be increased in frequency throughout the marker assisted selection. Identification of genotypes and their frequencies for goats CSN1S1 are important for selecting animals carrying the strong and mild alleles needed for improving milk quantity and quality for economic goat milk industry.

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REFERENCES

Abdelhamid, A.M., T.A.M. Ashmawy, Faten F Abou Ammou and Heba A. El-Sanafawy, 2012. Effect of dietary inclusion of whole sunflower seeds on feeding lactating Zaraibi goats: Milk production and composition as well as mammary gland histology and economic efficiency. Inter. J. Biotech. Res., 1, 6-19.

Addass, P. A., M. A. Tizhe, A. Midau, P. A. Alheri and M. M. Yahya, 2013. Effect of genotype, stage of lactation, season and parity on milk composition of goat, in Mubi, Adamawa State, Nigeria. Ann. Bio. Res. 4, 248-252.

Ahmed, Sahar, 2006. Identification of Egyptian goat αs-casien alleles using PCR-RFLP method. Int. J. Dairy Sci.1, 27-31.

Ahmed, Sahar and E.O. Othman, 2009. Genotyping Analysis of Milk Protein Genes in Different Goat Breeds Reared in Egypt. J. Genetic Eng. Biotech. 7, 33-39.

Anwar, M. M., T. A. Ramadan and T. A. Taha, 2012. Serum metabolites, milk yield and physiological responses during the first week after conditions kidding in Anglo-Nubian, Angora, Baladi, and Damascus goats under subtropical conditions. J. Anim. Sci. 90, 4795-4806.

Balia, F., M. Pazzola, M. L. Dettori, M.C. Mura, S. Luridiana, V. Carcangiu, P. Gianpiera and M.V. Giuseppe, 2013. Effect of CSN1S1 gene polymorphism and stage of lactation on milk yield and composition of extensively reared goats. J. Dairy Res. 80, 129-137.

Banda, J.W., J, Steinbach and H.P. Zerfas, 2006. Composition and yield of milk from non-dairy goats and sheep in Malawi. (http://www.fao.org).

Caroli, A., F. Chiatti, S. Chessa, D. Rignanese, P. Bolla and G. Pagnacco, 2006. Focusing on the goat casein complex. J. Dairy Sci. 89, 3178-3187.

Clark, S. and J.W. Sherbon, 2000. Genetic variants of alpha s1-CN in goat milk: breed distribution and associations with milk composition and coagulation properties. Small Rumin. Res. 38, 135–143.

Cosenza, G., R. Illario, A. Rando, P. Di Gregorio, P. Masina and L. Ramunno, 2003. Molecular characterization of the goat CSN1S101 allele, J. Dairy Res. 70, 237–240.

El-Saied, U.M., A.A. Raheem and I. Shaat, 2007. Precision of simplified milk recording for Zaraibi goats. Egyptian J. Anim. Prod., 44, 173-184.

Feligini, M., S. Frati, A. Cubric, V. Curik, A. Brambilla, P. Parma, I. Curik, G.F. Greppi and G. Enne, 2005. Caprine αs1casein polymorphism: characterisation of A, B, E and F variants by means of various biochemical and molecular techniques. Food Technol. Biotech. 432, 123–132.

Fernandez, M., H. Castillo- Juarez, J.R. Gonzalez, F.J. Fernandez, H Castaneda Vazquez and J.A. Saltijeral-Oaxaca, 2008. Somatic cell counts and quality of goat milk produced in the central region of Mexico. J. Daiy Sci. 2, 45-50.

Galal, S., Ferial Abdel Rasoul, M.R. Anous and I. Shaat, 2005. Small Ruminant Breeds of Egypt. In "Characterization of Small Ruminant Breeds in West Asia and North Africa, North Africa.", Ed. Luis Iňiguez, International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria, pp 141-193.

Habeeb, A.A.M., E.S. El-Gohary and S.A. El-Saadany, 2009. Improving milk yield and some physiology body functions of lactating Zaraibi goats under heat stress conditions of hot summer season using supplemental diet with ginger or curcumin. Zag. Vet. 37, 54-67.

Hamed, A., 2010. Genetics studies on Zaraibi goats. Ph. D. thesis, Fac of Agric. Al-Azhar Univ. Egypt.193

Kralickova, S., J. Kuchtik, R. Filipcik, T. Luzova and K. Sustova, 2013. Effect of chosen factors on milk yield, basic composition and somatic cell count of organic milk. Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis 1, 99–105.

Lynch, J.M., D.M. Barbano, M. Schweisthal and J.R. Fleming, 2006. Precalibration evaluation procedures for Mid-Infrared milk analyzers. J. Dairy Sci. 89, 2761–2774.

Marai, I.F.M., E.I. Abou-Fandoud, A.H. Daader and A.A. Abu-Ela, 2001. Association between marker gene alleles and doe traits in Nubian (Zaraibi) goats in Egypt. Annals of Arid Zone 40, 193-197.

Martin, P., M. Ollivier-Bousquet and F. Grosclaude, 1999. Genetic Polymorphism of Casein: A tool to investigate Casein micelle organized intl. Dairy J. 9, 164-171.

Martin, P., M. Szymanowska, L. Zwierzchowski and C. Leroux, 2002. The impact of genetic polymorphisms on protein composition of ruminant milks. Reprod. Nutr. Dev. 42, 433–459.

Mestawet, T. A., A. Girma, T. Adnoy, T. G. Devold, J. A. Narvhus and G. E. Vegarud, 2012: Milk production, composition and variation at different lactation stages of four goat breeds in Etiopia. Small Rumin. Res. 105, 176–181.

Miller, S.A., D.D. Dykes and H.F. Polesky, 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res. 16, 12-15.

Mowlem, A., 2005. Marketing goat dairy produce in the UK. Small Rumin. Res. 60, 207–213.

NRC, 2007. Nutrient Requirements of Small Ruminants (Goats), Cervids, and New World Camelids. Washington, DC.

Othman E. O. ,Samia A. El-Fikya, Nagwa A. Hassanb, Eman R. Mahfouza, Esraa A. Balabela, 2013. Genetic polymorphism detection of two α-Casein genes in three Egyptian sheep breeds. J. Genetic Eng. and Biotech. 11, 129-134.

Pazzola, M., M.L. Dettori, E. Pira, A. Noce, P. Paschino and G.M. Vacca, 2014. Effect of polymorphisms at the casein gene cluster on milk renneting properties of the Sarda goat. Small Rumin. Res. 117, 124–130.

Ramunno, L., G. Cosenza, A. Rando, A. Pauciullo, R. Illario, D. Gallo, D. Di Berardino and Masina, P. , 2005. Comparative analysis of gene sequence of goat CSN1S1 F and N alleles and characterization of CSN1S1 transcript variants in mammary gland. Genetics 345, 289–299.

Ramunno, L., G. Cosenza, M. Pappalardo, E. Longobardi, D. Gallo, N. Pastore, P. Digorio and A. Rando, 2001b. Characterization of two new alleles at the goat CSN1S2 locus. Anim. Genetics 32, 264–268.

Ramunno, L., G. Cosenza, M. Pappalardo, N. Pastore, D. Gallo, P. Di Gregorio and P. Masina, 2000. Identification of the goat CSN1S1F allele by means of PCR-RFLP method. Animal Genetics 31, 342-343.

Rando, A., L. Ramunno and P. Masina, 2000. Mutations in casein genes. Zoot. Nutr. Anim. 26, 105-114.

Roncada, P., A. Gaviraghi, S. Liberatori, B. Canas, L. Bini, G.F. Greppi, 2002. Identification of caseins in goat milk. Proteomics 2, 723-726.

Rout, P.K., A. Kumar, A. Mandal, D. Laloe, S.K. Singh and R. Roy, 2010. Characterization of Casein Gene Complex and Genetic Diversity Analysis in Indian Goats. Anim. Biotech. 21, 122–134.

Sanger, F., S. Nicklen and A.R. Coulson, 1977. DNA sequencing with chain terminating inhibitors. Proc. Natl. Acad. Sci. U. S. A. 74, 5463–5467.

SAS., 2000. SAS/STAT® 9.2 User's Guide, 2nd ed. SAS Institute Inc, Cary, NC, USA.

Smith, E. B., D. M. Barbano, J. M. Lynch and J. R. Fleming, 1993a. Performance of homogenizers in infrared milk analyzers: A survey. J. Dairy Sci. 76, 1033–1041.

Strzalkowska, N., A. Jozwik, E. Bagnicka, J. Krzyzewski, K. Horbanczuk, B. Pyzel, , D. Sloniewska and J. O. Horbanczuk, 2010. The concentration of free fatty acids in goat milk as related to the stage of lactation, age and somatic cell count. Ani. Sci. Papers and Reports 28, 389–395.

Sztankoova, Z., V. Matlova, J. Kyselova, O.M. Jandurova, J. Riha and C. Senese, 2006. A new allele specific polymerase chain reaction method (AS-PCR) for detection of the goat CSN1S101 allele. Small Rumin. Res. 66, 282–285.

Teleb, Doaa F., U. M. El-Saied, A. A. Sallam, Azza M. El-Baz and A.M. Hussein, 2009. Effect of Using Echinacea Extract as Immuno-stimulating Additive on milk yield Traits, Immunity and Udder Health of Zaraibi goats, Egyptian J. Sheep & Goat Sci., 4, 33-53

Vacca G. M., M. L. Dettori, V. Carcangiu, A.M. Rocchigiani and M. Pazzola, 2010. Relationships between milk characteristics and somatic cell score in milk from primiparous browsing goats. Ani. Sci. J. 81, 594–599.

Vacca, G.M., H. Ouled Ahmed Ben Ali, V. Carcangiu, M. Pazzola and M.L. Dettori, 2009. Genetic structure of the casein gene cluster in the Tunisian native goat breed. Small Rumin. Res. 87, 33–38.

Zullo, A., C.M.A. Barone, L. Chianese, P. Colatruglio, M. Occidente and D. Matassino, 2005. Protein polymorphisms and coagulation properties of Cilentana goat milk. Small Rumin. Res. 58, 223–230.

Table (1): Primer sequences used for detecting different alleles of CSN1S1 gene

Allele	Primer name	Sequence (5'-3')	Annealing temperature (ºC)	Reference
A, B, C and D	CSN1S1-F	TTCTAAAAGTCTCAGAGGCAG	59	**Ramunno *et al*., 2000**
	CSN1S1-R	GGGTTGATAGCCTTGTATGT		
A and 0	CSN1S1A-R	CTCAGCACTTTTGGGAACAAT	59	**Cosenza *et al*., 2003**
	CSN1S101-R	CCTCTCCTTTAACTTTCCC		
	CSN1S1A/0-F	AACGTGCCCCAGCTG		
B and E	CSN1S1B-F	CAACCTCAAATTGAAGGCACT	54	**Feligini *et al*., 2005**
	CSN1S1E-F	TGGTGTTTTTCTTTCTGGCTTA		
	CSN1S1B/E-R	CAAGCTCTTAGGACAATTTCACTT		

Table (2): Allele and genotype frequencies for CSN1S1 gene in Zaraibi goat.

Alleles		**Genotypes**		
Type	Frequencies (%)	Type	No. of Animals	Frequencies (%)
A	17.76	AA	16	10.53
B	27.63	BB	28	18.42
C	26.97	CC	28	18.42
D	27.63	DD	27	17.76
		AC	16	10.53
		AD	6	3.95
		BC	7	4.61
		BD	21	13.82
		CD	3	1.97
		Total	152	100

Table (3): LSM (±SE) of daily milk yield and composition in Zaraibi goats as affected by CSN1S1 genotypes.

Genotype	**No**	**Daily milk yield**	**Milk Composition (%)**				
			Fat	**Protein**	**Lactose**	**Total solid**	**Solid not-fat**
AA	14	1.30 ± 0.10^{b}	2.95 ± 0.22^{b}	2.55 ± 0.17^{b}	3.92 ± 0.20^{a}	9.65 ± 0.52^{b}	7.16 ± 0.24^{b}
BB	23	1.29 ± 0.08^{bc}	3.27 ± 0.17^{ab}	2.67 ± 0.13^{b}	4.17 ± 0.16^{a}	10.40 ± 0.42^{ab}	7.54 ± 0.19^{b}
CC	25	1.33 ± 0.07^{b}	3.35 ± 0.15^{ab}	2.70 ± 0.11^{b}	3.97 ± 0.14^{a}	10.12 ± 0.36^{b}	7.37 ± 0.16^{b}
DD	15	1.07 ± 0.09^{b}	3.28 ± 0.21^{ab}	2.98 ± 0.16^{b}	3.96 ± 0.19^{a}	10.63 ± 0.50^{b}	7.64 ± 0.23^{b}
AC	12	1.25 ± 0.11^{c}	3.27 ± 0.24^{ab}	2.82 ± 0.18^{b}	3.96 ± 0.21^{a}	10.36 ± 0.56^{ab}	7.48 ± 0.26^{b}
AD	2	2.00 ± 0.18^{a}	3.96 ± 0.47^{a}	3.84 ± 0.35^{a}	4.16 ± 0.42^{a}	12.30 ± 1.11^{a}	8.71 ± 0.51^{a}
BD	18	1.41 ± 0.08^{b}	3.43 ± 0.19^{ab}	2.80 ± 0.14^{b}	3.81 ± 0.17^{a}	10.33 ± 0.45^{b}	7.31 ± 0.21^{b}
Significance		*S (p<0.05)	S (p<0.05)	S (p<0.05)	**NS	S (p<0.05)	S (p<0.05)

a and b= Means with the different letters are significantly different.

*S= significantly different **NS=non significantly different No. = number of records

Table (4): LSM (±SE) of daily and total milk yield in Zaraibi goats as affected by lactation time and parity.

Item	No.	Daily milk yield (kg/ day)	Total milk yield (kg)
Time			
7	50	2.65±0.24[a]	45.67±19.47[g]
14	50	2.73±0.24[a]	62.76±19.47[f]
30	50	2.47±0.24[b]	97.71±19.47[e]
60	49	2.27±0.24[c]	157.53±19.47[d]
90	48	2.01±0.24[d]	210.45±19.48[c]
120	45	1.56±0.24[e]	249.74±19.47[b]
210	37	1.01±0.24[f]	317.69±19.59[a]
Significance		*S (p<0.0001)	S (p<0.0001)
Parity			
1	41	1.22±0.09[d]	84.60±7.52[d]
2	107	1.39±0.07[c]	100.19±5.53[d]
3	46	1.93±0.05[b]	148.00±4.39[c]
4	54	1.97±0.05[b]	145.59±4.01[b]
5	41	1.93±0.06[b]	140.93±5.36b[c]
6	14	2.06±0.17[ab]	163.87±13.37[a]
7	26	2.00±0.22[a]	163.67±17.55[ab]
Significance		S (p<0.0001)	S (p<0.0001)

a and b= Means with the different letters within a subclass of same column are significantly different.

*S= significantly different. No. = number of records.

Table (5): LSM (±SE) of milk composition in Zaraibi goats as affected by lactation time and parity.

Item	No.	Milk Fat	Protein	Lactose	Total Solid	Solid not-fat
Time						
90	42	3.91±0.16[a]	2.84±0.12[b]	4.11±0.15[a]	11.21±0.39[a]	7.65±018[a]
120	37	2.56±0.17[c]	2.60±0.13[c]	4.22±0.16[a]	9.58±0.41[b]	7.52±0.19[a]
210	30	3.60±0.18[b]	3.28±0.13[a]	3.66±0.16[b]	10.82±0.42[a]	7.63±0.19[a]
Significance		*S (p<0.0001)	S (p<0.0001)	S (p<0.002)	S (p<0.0003)	**NS
Parity						
1	11	3.45±0.24[a]	2.74±018[a]	4.20±022[a]	10.72±0.57[a]	7.64±0.26[a]
2	37	3.32±0.16[a]	2.69±0.12[a]	4.26±0.15[a]	10.53±0.39[a]	7.65±0.18[a]
3	17	3.28±0.20[a]	2.79±0.15[a]	3.95±0.18[a]	10.22±0.48[a]	7.45±0.22[a]
4	17	3.24±0.18[a]	2.88±0.13[a]	3.85±0.16[a]	10.20±0.42[a]	743±0.20[a]
5	14	3.35±0.24[a]	2.99±0.18[a]	3.92±0.22[a]	10.59±0.57[a]	7.62±0.26[a]
6	3	3.73±0.39[a]	3.05±0.30[a]	3.99±0.36[a]	11.05±0.94[a]	7.73±0.43[a]
7	10	3.13±0.22[a]	3.20±0.16[a]	3.78±0.20[a]	10.47±0.52[a]	7.68±0.24[a]
Significance		NS	NS	NS	NS	NS

a and b= Means with the different letters are significantly different.
*S= significantly different. **NS= non significantly different.
No. = number of records.

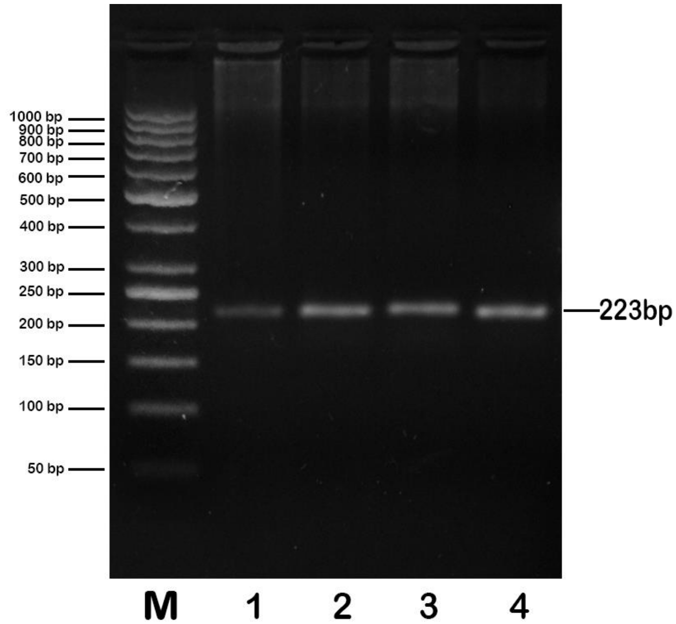

Figure (1): The electrophoretic pattern of PCR products of CSN1S1. M: 50 bp ladder. Lanes 1–4: 223 bp PCR product of CSN1S1.

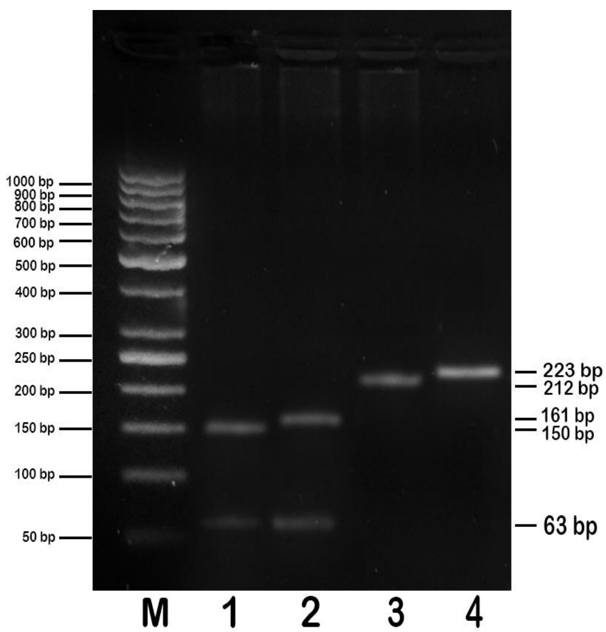

Figure (2.a): The electrophoretic pattern obtained after digestion of PCR amplified goat CSN1S1 products with XmnI. M: 50 bp ladder marker. Lanes 1, 2, 3 and 4: genotype (AA, BB, CC and DD, respectively).

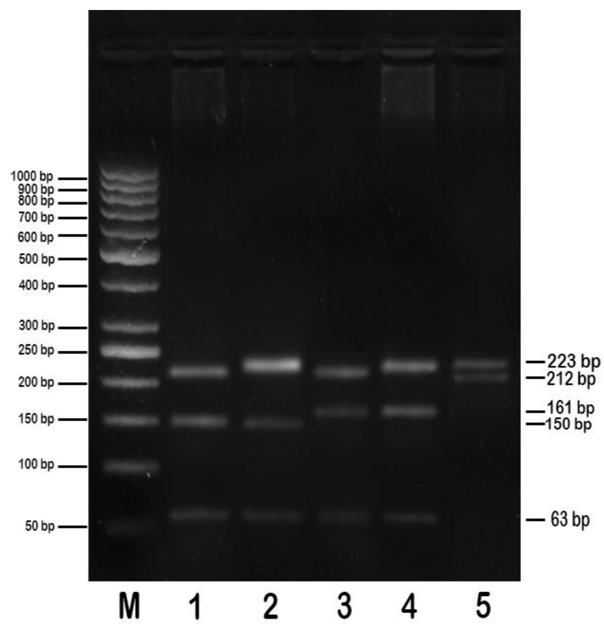

Figure (2.b): The electrophoretic pattern obtained after digestion of PCR amplified goat CSN1S1 products with XmnI. M: 50 bp ladder marker. Lanes 1, 2, 3, 4 and 5: heterozygous genotype (AC, AD, BC, BD and CD, respectively).

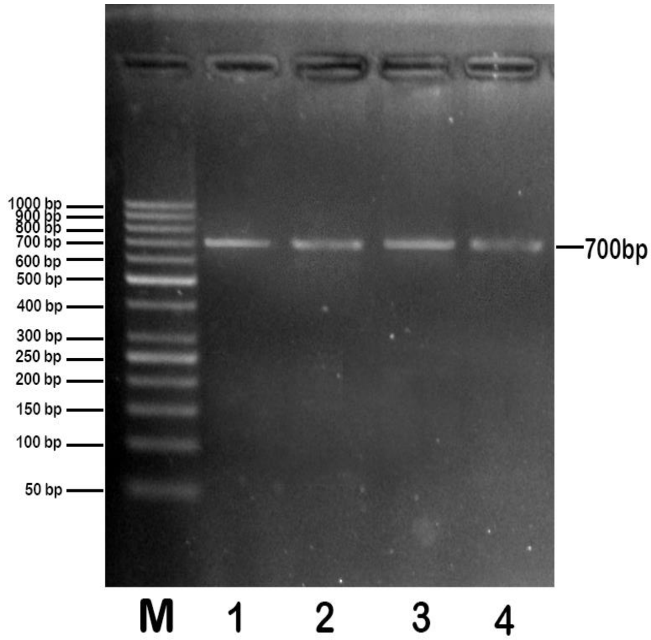

Figure (3.a): The electrophoretic pattern obtained after AS-PCR for CSN1S1 gene using CSN1S1A/0-F primer. M: 50 bp ladder marker.
Lane 1, 2, 3 and 4: 700 bp characterized for A allele.

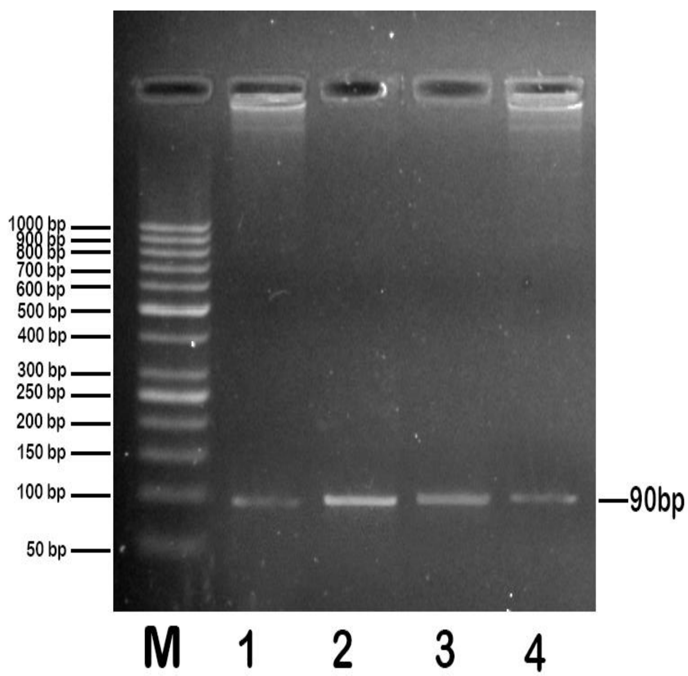

Figure (3.b): The elecrophoretic pattern obtained after AS-PCR for CSN1S1 gene using CSN1S1B/E-F primer M: 50 bp ladder marker.
Lane 1, 2, 3 and 4: 90 bp characterized for B allele.

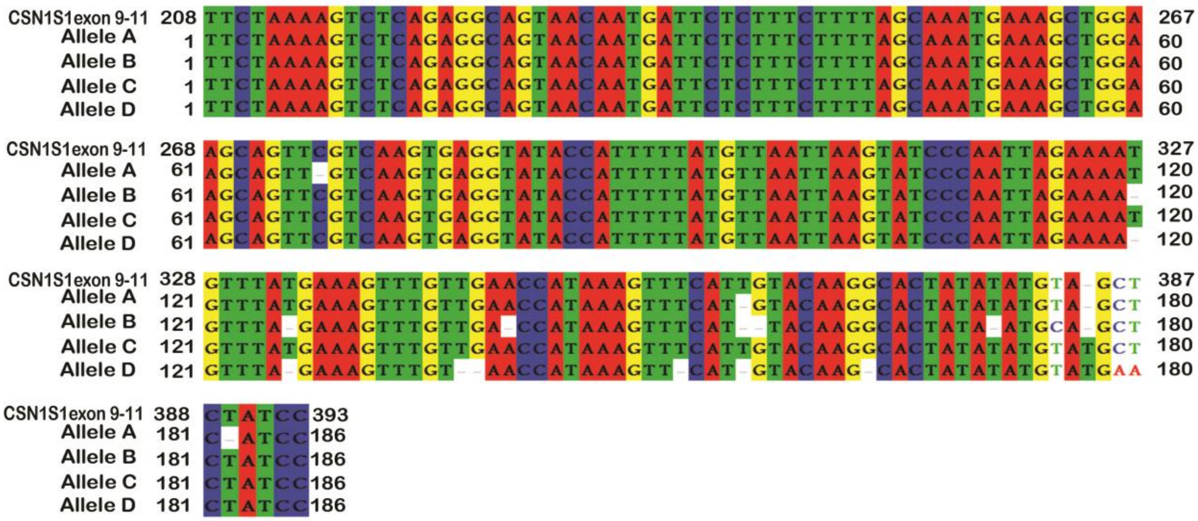

Figure (4): Alignment between sequences of the four detected alleles of the CSN1S1 gene in Zaraibi goats and their corresponding region from GenBank.

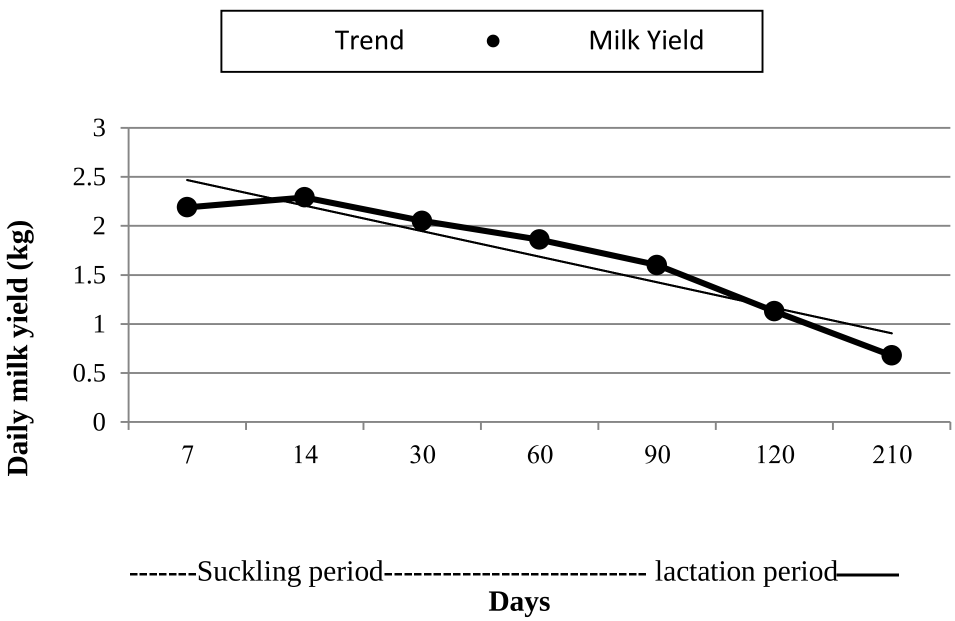

Figure (5): Average daily milk yield (kg) in Zaraibi goats during lactation period.

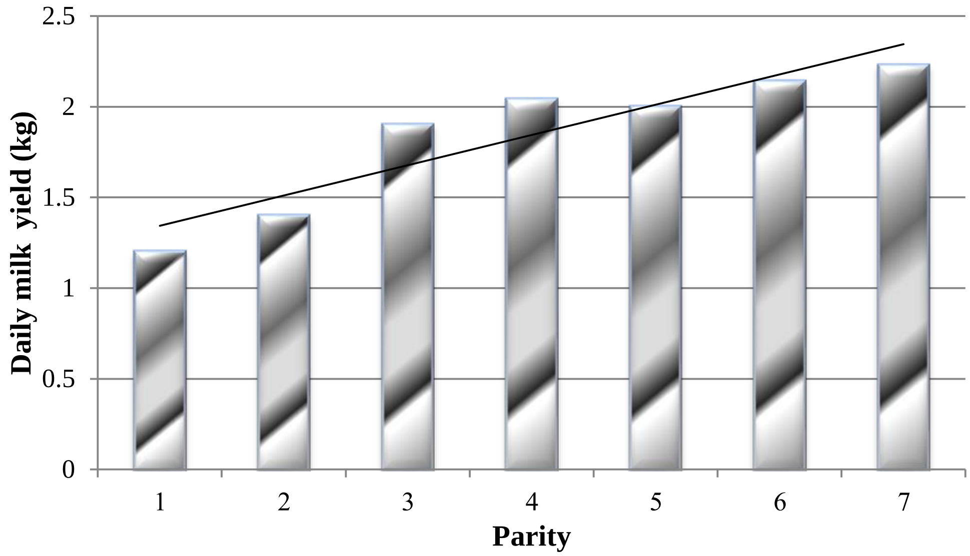

Figure (6): Average daily milk yield (kg) in Zaraibi goats as affected by parity.

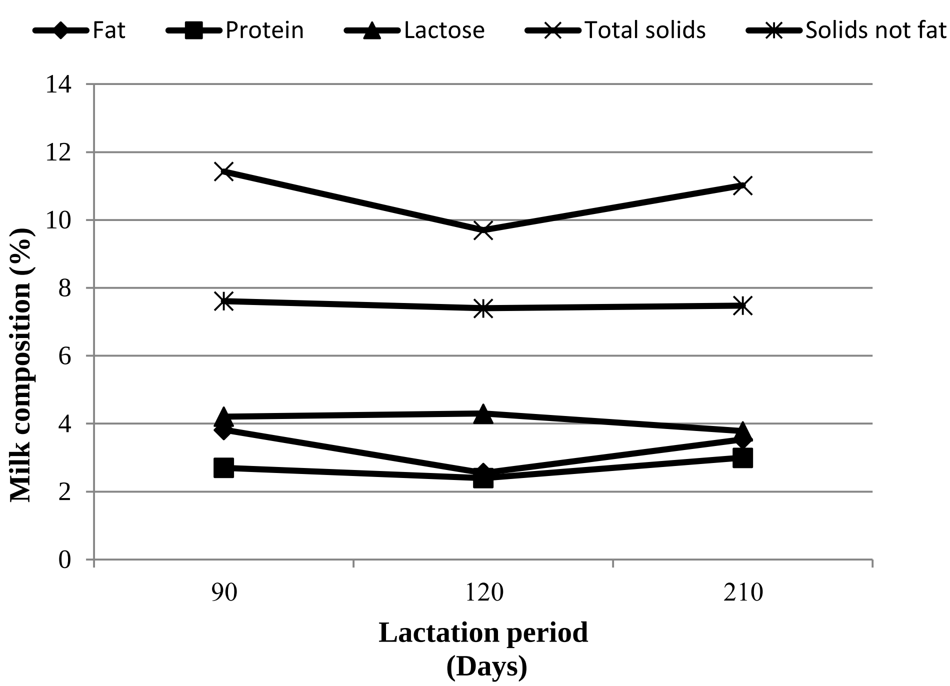

Figure (7): Average milk composition (%) in Zaraibi goats as affected by lactation time.

Role of genetic variation of αs1- casein, lactation time and parity on milk production and composition of Zaraibi goats

الملخص العربي

دور الاختلافات الوراثية لـ αs1- casein و وقت الحليب و عدد مرات الولادة على إنتاج ومكونات اللبن في الماعز الزرايبي

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تهدف هذه الدراسة إلي التعرف على مدى تأثير التباين الوراثي لـCSN1S1 و وقت الحليب و عدد مرات الولادة على الصفات الكمية والنوعية للبن. تم استخدام عدد 165 من الماعز الزاريبي (105 من الجداء و 7 ذكور و 53 إنثى) وذلك بعد موسم الولادة فى فبراير/مارس 2013.

أظهرت نتائج التفريد الكهربائى لناتج هضم تفاعل البلمرة لـCSN1S1 بإنزيم ***XmnI*** وجود عدد 9 طرز جينية، منهم عدد 4 طرز جينية متماثلة (AA, BB, CC, DD) و خمس طرز خليطة (AC, AD, BC, BD, CD). تأتى هذه الـ 9 طرز الجينية من 4 أليلات منهم عدد ثلاثة أليلات قوية (A, B, C) ذات تكرار (17.76، 27.63 ، 26.97%، على التوالى)، وعدد 1 أليل متوسط (D) تكراره 27.63%. وقد كانت الطرز الجينية BB وCC هم الأكثر تواجداً وبتكرار متساوى (18.42%)، يليهم DD (17.76%)، BD (13.82%)، AA (10.53%)، AC (10.53%)، BC (4.61%)، AD (3.95%) و CD (1.97%) .

أوضحت نتائج هذه الدراسة ان متوسط انتاج اللبن اليومي ونسبة الدهون والبروتين والمواد الصلبة والمواد الصلبة الغير دهنية تتأثر معنويا بالطرز الجينية لـCSN1S1. أظهرت إناث الماعز الزاريبي التى تحمل الطرز الجيني AD أعلى انتاج يومي من اللبن و كذلك أعلى نسبة من الدهون والبروتين والمواد الصلبة والمواد الصلبة الغير دهنية فى اللبن. كما أظهرت نتائج تعداد التتابعات الموجودة بالجزء محل الدراسة من الجين ظهور حذف للنيوكليوتيدة C من الأليل A مما أدى إلى استبدال التتابع TT<u>C</u> بـ TT<u>G</u> وتكوين الحمض الأميني Leucine بدلا من Phenyl alanine فى الناتج النهائى للبروتين فى حين لم يظهر أي تباين وراثي في النيوكليوتيدات فى المنطقة من 1-274 من الجزء محل الدراسة من الأكسون 9 ولذلك يعتبر هذا الجزء موحدا بين الأليلات (A, B, C, D).

أظهرت النتائج إن متوسط إنتاج اللبن اليومي كان أعلى معنويا في الاسبوع الاول بعد الولادة ويصل لأعلى مستوى في الأسبوع الثاني، ثم يبدأ في الإنخفاض حتى نهاية موسم الحليب. كما تم تسجيل أعلى انتاج يومي للبن في الإناث ذات الولادة السادسة والسابعة والإنتاج الأقل في الإناث ذات الولادة الأولى والثانية. و كان مستوى الدهون والمواد الصلبة الأعلى في اليوم 90 (نهاية فترة الرضاعة) عن مستواه في خلال فترة الحلب (يوم 120 و 210). كما كان أعلى تركيز للبروتين في اليوم 210 (نهاية فترة الحليب) فى حين سجل اللاكتوز أقل تركيز.

نستخلص من هذه النتائج أهمية دراسة التباين الوراثي لـCSN1S1 في الماعز الزرايبي حتى يمكن التعرف على الطرز الجينية المفضلة و المرتبطة بإنتاج اللبن و محتواه من الدهون والبروتين.

لذا من الأفضل توجيه برامج التربية لإختيار الحيوانات التي تحمل الاليلات القوية و المتوسطة المرتبطة بنسبة البروتين و الدهون فى اللبن بهدف تنمية صناعات الألبان فى الماعز.