American-Eurasian J. Agric. & Environ. Sci., 6 (2): 188-195, 2009
ISSN 1818-6769

Effect of Extensive and Intensive Feeding Regime on Goat Milk Composition Affected by κ-casein Polymorphism Gene

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Abstract: Goat milk samples (129) were collected from 43 farms, 3 goats each farm and employed to study the effect of feeding regime on milk composition and quality affected by κ-casein polymorphism gene (CSN3 variants). Samples testing positive in a bacteriological examination were excluded from the study. The treatment arrangement was a 3 x 2 factorial design, with three feeding regimes, at different locations in the West Bank (Hebron, HB; Jericho, JR; and Ezerriah, EZ) and two CSN3 genotype alleles (AB and BB). The semi-extensive feeding regimes with low quality pasture plus high or low concentrate supplement in HB and JR, respectively and an intensive regime in EZ with moderate hay and concentrate supplement were considered. A method of characterization of casein A and B variants was developed using PCR – RFLP DNA assay. The genotype BB predominated (82.9%) the CSN3 gene, followed by the AB heterozygote (15.4%), whereas AA genotype was found in only 1.7% of samples. The interaction between feeding regime and genotype allele was significant for milk density and concentrations of protein, lactose and solids-non-fat. Levels were similar among feeding regimes for the BB allele but for the AB heterozygote were greater in HB than in JR ($P < 0.05$), while EZ had intermediate values. These results suggest that effects of feeding regime on milk composition are controlled by gene background and the CSN3 genotype alleles may serve as a marker for animal selection to alter milk composition as relating to milk industries.

Key words: Goat · Feeding regime · κ-casein polymorphism · Milk composition

INTRODUCTION

Goats play an important role in the economic and social life in the world, particularly in arid and semi-arid areas. Goats are more adaptable to environmental conditions prevailing in dry climatic regions compared with other ruminant species; therefore, goat milk is considered essential to the well being of people in developing countries of Africa and Asia. Milk and dairy products such as cheese and yogurt are valuable sources of nutrients i.e., minerals, vitamins, protein and fat [1, 2]. Approximately 300 breeds of goats (*Capra hircus*) in the world have been described by Porter [3], reflecting selection for various characteristics and adaptations to different climatic conditions. The indigenous goat breed in the Middle East is the Baladi, which is a dual-purpose breed used for meat and milk. However, its milk production is rather poor. The Shami breed originated in Syria and Lebanon and is high in milk production relative to Baladi goats. Therefore it has been a common practice to crossbreed Baladi and Shami goats to enhance milk production. Goats raised by Palestinians are either pure Baladi or Baladi x Shami crosses.

Goat milk production is considered an important economic activity in the Middle East region in which its composition and quality can be affected by different factors such as climate or environmental condition, animal genetics and feed resources [2]. The adaptation of black Bedouin goats to desert condition was reported by Shkolnik *et al.* [4] and Maltz and Shkolnik [5], in which nutrient metabolism and milk yield and content were affected by water scarcity and arid environment. However, milk protein is comprised of 71% casein, 22% whey proteins and 7% non-protein nitrogen [6]. Casein is a rapidly evolving gene family. Casein micelles are made of α-casein $_{s-1,\ s-2,}$ β-casein and κ-casein. These

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genes are coded by CSN1S1, CSN1S2, CSN2 and CSN3 loci, respectively [7]. The κ-casein protein, constituting 15% of total casein, plays an important role in stabilization, formation and aggregation of casein micelles, making it most important in processing milk into cheese [8-10]. A high degree of polymorphism was found at the κ-casein locus in goats and at least 16 alleles are known so far [11, 12]. It is recommended to incorporate milk protein alleles as criteria into dairy breeding programs because of their economic important in milk products

Scarce information is available about effects of feeding management and κ-casein polymorphism gene on goat milk composition and quality in the West Bank. Therefore, the aim of current research was to study effects of different feeding regime at different areas in the West bank on milk composition affected by genotype of the κ-casein gene (CSN3 variants).

MATERIALS AND METHODS

Animal Managements and Treatments: Forty-three goat farms on three different feeding regimes at different areas in the West Bank were employed to study the effect of feeding management, k-casein polymorphism gene and their interaction on milk composition and quality. Milk was daily sampled through three consequective days and pooled in one sample per each goat. Three milk samples from three goats per each farm were obtained in which 11, 19 and 13 farms were used in Hebron (HB; approximately, 30 km south of Jerusalem), Jericho (JR; approximately 25 km northeast of Jerusalem) and Ezerriah (EZ; approximately, 3 km southeast of Jerusalem), respectively. A total of 129 milk and 36 blood samples were collected in which milk samples were obtained during the fifth or sixth weeks of lactation. However, after excluded samples of the infected animals only 32, 50 and 35 milk samples were considered in this study for HB, JR and EZ, respectively. However, all animals were healthy and ranged in age from 3 to 5 years. According to the Palestinian Authority Ministry of Agriculture of 2007, number of goats in are 80.000 and 66.000 for HB and JR, respectively, but no records were found for EZ.

The treatment arrangement was a 3 x 2 factorial design, with three feeding regimes at different three areas and two genotype alleles (CSN3 variants, AB and BB). The feeding regimes were semi-extensive feeding regimes in HB and in JR and an intensive regime in EZ. Goats in HB rely primarily on grazing of low-quality forage of Bermuda grass (*Cynodon dactylon*) and daily supplemented with concentrate feed (Table 1) based on 1.7% of live body weight (HB, treatment 1), while goats in JR depend on grazing similar low quality forage as in HB one but with less supplemental concentrate feed (1.2% of live body weight). However, goats in EZ were housed in barns and given free access of moderate quality hay and supplemental concentrate based on 1.7% of live body weight (EZ, treatment 3).

Table 1: Chemical composition of feed supplement, alfalfa hay and Bermuda grass

Chemical Composition	Feed Supplement	Alfalfa Hay	Bermuda Grass
Dry matter, %	88	89	36
Organic matter, %	93	88	89
Crude protein, %	16	14.8	14
Crude fiber, %	7	23.5	30
Ether extract, %	3	2.6	1.3

Analytical Procedure

1.bacteriological Examination: Fresh milk samples were examined on-site with the California Mastitis Test (CMT; Kerba Test, Eurofarm, Buchbach Germany). Samples testing positive were subjected to bacteriological test according to accepted standards described by Hogan *et al.* [13]. Colonies suspected to be *Staphylococci* were tested for coagulase using staphylase test kit (OXOID, X6794A, Basingstoke, UK). Then plates were subjected to API STAPH – IDENT, 32 staph kit (Biomerieux S.A., 69280 Marcy-1'Etoile, France). *Streptococcus spp.* was identified using Streptococcal grouping kit (OXOID, X6793A, Basingstoke, UK).

2.Feed Analysis: In concentrate feed, dry matter content was determined by oven drying at 105°C for 24 hours, while forage samples were dried at 65°C for 48 h. Organic matter was obtained by ashing at 550°C for 8 h. Total N content and crude fiber of feed was determined following the Association of Official Analytical Chemists [14].

3.Milk Analysis: Milk samples were analyzed for fat, protein, total solids, solids-non-fat (SNF) and lactose, using a Lactoscan-90 milk analyzer (BOECO, Hamburg, Germany) calibrated with goat milk standards, in which samples were analyzed in duplicate.

DNA Extraction from Milk and Blood and PCR-RFLP Analysis: Specific primers were designed on the basis of the caprine sequence of GenBank accessions (AF434987, X60763) of allele A and allele B (AF434988, AF485340) to

analyze the CSN3 that identifies the A (G/G homozygote) and B (A/A homozygote) alleles, covering the sequence and containing the mutation site G/A at position 471 in exon 4. In addition, we have searched for restriction sites in these sequences using the NEBcutter V2.0 program (http://tools.neb.com/NEBcutter2/index.php).

DNA was obtained from 36 blood and 117 milk samples using the Master Pure DNA Purification Kit (Ambion, MG71100, Madison WI USA) as recommended by manufacturer. The DNA concentration was evaluated by using Nano-drop spectrophotometer (ND-1000) (Thermo Fisher Scientific, Wilmingston, DE, USA). A 256 bp fragment containing exon 4 of goat CSN3 gene was amplified by the PCR. The reaction was carried out using 1 uM of each primer F-CSN3 (5' ATA CTG TGC CTG CCA AGT CC 3') and R-CSN3 (5' GGG CTG TGT TGG TCT CAG AT 3'). The PCR reaction was carried out in total 25 µl reaction using PCR-Ready Supreme mix (Syntezza Bioscience, Jerusalem) in a Gene Amp PCR-system 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA), using an initial denaturation at 95°C for 5 min, then 35 cycles of denaturation (95°C for 30 sec), annealing (55°C for 45 sec) and polymerization (72°C for 45 sec). The cycling was followed by a final extension step at 72°C for 10 min. Ten µl of the PCR product were analyzed on 3% agarose gel by electrophoresis at 120 volts. The PCR was considered positive when a band with 256 bp was observed and negative control with no DNA included in each reaction test.

A 15 µl of the PCR products were digested for 1.5 h at 37°C in the appropriate buffer. The restriction fragments were separated in 3.5% agarose gel (FMC BioProducts, Rockland, ME, USA) by electrophoresis in 1X Tris –acetate-EDTA. Banding profiles were observed by UV light and the fragment sizes determined using DNA molecular marker *Hinf* 174 (Promega, Madison, WI USA). Negative controls for extracted DNA and PCR analysis were included.

Sequencing: PCR products were purified using the purification Kit (Qiagen) and sequenced as proposed by Reale *et al.* [15] to determine both A and B alleles, using the ABI Prism BigDye Terminator v1.1 Cycle Sequencing Ready Reaction Kit (Applies Biosystems, Foster City, CA).

Statistical Analysis: All milk samples were subjected for milk composition and genotyping analysis. The variant allele BB genotype showed the highest frequency and was predominant among all study sites (i.e., 82.9%). The heterozygote genotype AB was present at 15.4% and the genotype AA occurred less frequently (i.e., 1.7%). Because of the low frequency of AA, animals with AA genotype were excluded from the statistical analysis. In addition, samples that had a positive bacteriological test were also excluded from the analysis. Data of 117 milk samples were statistically analyzed with the GLM procedure of SAS statistical package (Version 8.01). Fixed effects in the model were feeding regime, Allele genotype and their interaction.

RESULTS

Bacteriology Test: As noted earlier, all animals appeared healthy without signs of clinical mastitis or other illness. Nonetheless, 38 samples were CMT positive, of which intramammary infections accompanying with or without subclinical symptoms were observed in 12 samples. Pathogenic distribution was as follows: 41.6% *Staphylococcus aureus*, 16.6% *Escherichia coli,* 33.3% *Klebsiella* and 8% Streptococcal group D but no clinical abnormalities were shown among tested goats. According to farmer's observations, these animals had low milk yield and were not subjected to statistical analyses. Moreover, this observation is in agreement with findings of Leitner *et al.* [16] for lower milk yield by infected than uninfected mammary glands of goats.

Feeding Managements: The means of main effect are presented in Table 2 when the feeding regime x κ-casein polymorphism allele interaction was nonsignificant

Table 2: Effect of feeding regime and k-casein polymorphism gene on goat milk composition at different areas in the West Bank

	κ-casein Allele[2]			Treatment[1]			
Item	AB	BB	SEM	HB	JR	EZ	SEM[3]
Fat, %	3.76	4.08	0.204	3.57	3.90	4.28	0.268
SNF*, %							
κ-casein AB				9.66^{a}	8.16^{b}	8.84^{ab}	0.212
κ-casein BB				8.51^{b}	8.39^{b}	8.17^{b}	
Density, g/ml							
κ-casein AB				1.035^{a}	1.029^{c}	1.033^{ab}	0.0008
κ-casein BB				1.030^{bc}	1.030^{c}	1.029^{c}	
Protein, %							
κ-casein AB				4.66^{a}	3.98^{b}	4.24^{ab}	0.097
κ-casein BB				4.13^{b}	4.03^{b}	4.03^{b}	
Lactose, %							
κ-casein AB				4.06^{a}	3.38^{b}	3.72^{ab}	0.093
κ-casein BB				3.57^{b}	3.55^{b}	3.46^{b}	
TS*, %	12.64	12.43	0.234	12.07	12.99	12.56	0.307

$^{a-c}$ Means within a row with different superscripts differ *(P<0.05)*.

[1]Treatments: HB, JR = Extensive feeding regimes in Hebron and Jericho with high and low concentrate supplent, respectively; EZ = Intensive feeding regime in Ezerriah.

[2] κ-casein alleles: AB = κ-casein AB genotype; BB= κ-casein BB genotype. *SNF= Solid non fat; TS=Total solid. [3]SEM = standard error of the mean.

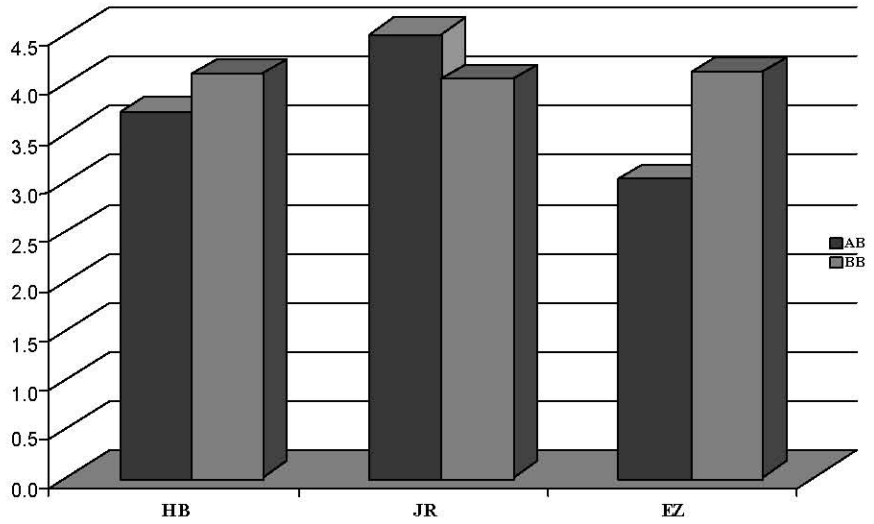

Fig. 1: Effect of different feeding regimes on fat milk composition in goats at different areas in the West Bank. HB and JR is the extensive feeding regimes in Hebron and Jericho with high and low concentrate supplement, respectively; EZ is the intensive feeding regime in Ezerriah. AB = κ-casein AB genotype; BB = κ-casein BB genotype

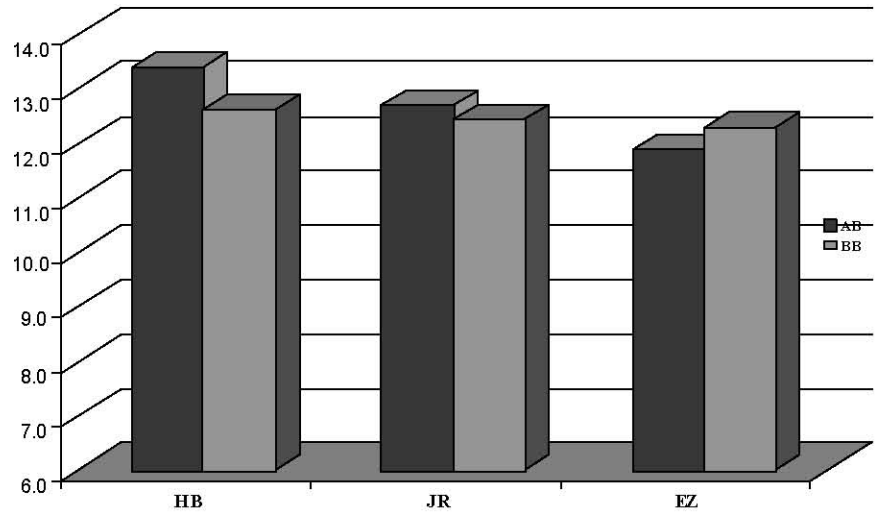

Fig. 2: Effect of different feeding regimes on total solid milk composition in goats at different areas in the West Bank. HB and JR is the extensive feeding regimes in Hebron and Jericho with high and low concentrate supplement, respectively; EZ is the intensive feeding regime in Ezerriah. AB = κ-casein AB genotype; BB = κ-casein BB genotype.

($P > 0.05$). Interaction means are given in Fig. 1 and 2 regardless of significance of the interaction. Milk composition was similar between EZ and JR, but a number of differences between HB and the other two treatments were noted with SNF, lactose and protein ($P < 0.05$). Milk density was significantly ($P < 0.05$) higher with HB and EZ compared to JR (1.033, 1.031 and 1.029 g/ml, SE = 0.0006, respectively). Otherwise, fat and total solid (TS) content were not affected by treatments or allele (Table 2, Figures 1 and 2). Levels of SNF, protein and lactose were greater ($P < 0.05$) for AB than those for BB genotype (8.89 vs. 8.36%, SE = 0.124; 4.29 vs. 4.06%, SE = 0.057; and 3.72 vs. 3.52%, SE = 0.054 for SNF, protein and lactose, respectively (Table 2). Similar trend was shown with milk density (1.032 vs. 1.030 g/ml, SE = 0.0005, $P < 0.05$, respectively). The interaction between feeding system and genotype was significant for concentrations of SNF, protein, lactose and density ($P < 0.05$; Table 2).

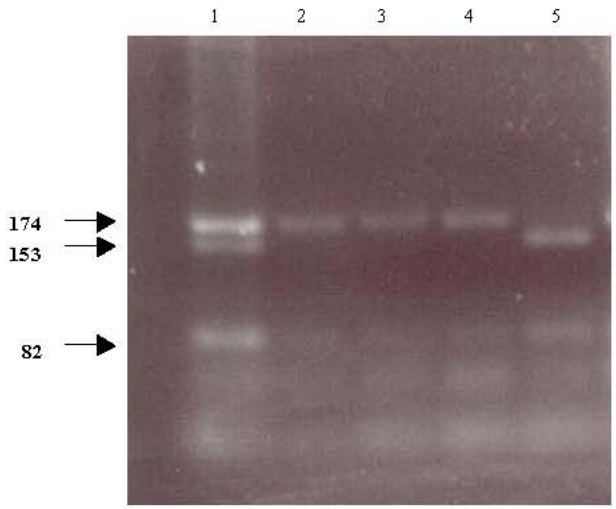

Fig. 3: PCR RFLP products of κ-casein alleles for goat milk samples. 1: genotype AB, 2-4: genotype BB, 5: genotype AA.

Fig. 4: Multiple alignment for the sequence of the κ-casein alleles for goat milk samples using new designed primers. Goat 1 with AA, goats 3, 4, 5 and 6 with BB and goats 2 with AB genotype.

Levels were similar among feeding systems for the BB allele but for the AB heterozygote were greater in HB than in JR ($P < 0.05$), while EZ had intermediate values. There was a tendency ($P < 0.10$) for an interaction in fat concentration between feeding system and genotype. For the BB genotype, fat concentration was similar among feeding systems (4.11, 4.09 and 4.04%, SE = 0.349, for EZ, HB and JR, respectively). But, for the AB genotype fat concentration was 3.03, 3.71 and 4.53%, SE = 0.349, for EZ, HB and JR, respectively.

PCR-RFLP Analysis and Sequencing: Identification of A and B alleles of CSN3 was performed by amplification of a fragment of 256 bp located on exon 4, followed by digestion analysis. Both *HaeIII* and *BsmFI* restriction analysis differentiated between allele A and B producing distinguishable fragment sizes for each variant. The *HaeIII* enzyme was applied to ensure digestion and that no inhibition occurred during the restriction analysis. Digestion with both *BsmF1* and *Hae III* restriction enzymes revealed three genotypes. HaeIII digested the 256 bp product of B allele (A/A homozygote) producing two fragments with sizes; 173 and 82 bp, while both *HaeIII* and *BsmfI* digested A allele (G/G homozygote) with three fragments (153, 83, 21 bp) and four fragments for AB allele (G/A heterozygote, 174, 153, 82, 21bp) as shown in Fig. 3. These findings were confirmed by sequencing (Fig. 4).

Table 3: Frequency of the κ-casein alleles in goat milk on different feeding regime at different areas in the West Bank.

Feeding	κ-casein allele[2]			
Regime[1]	AA	AB	BB	Total
HB	0	4	28	32
JR	0	10	40	50
EZ	2	4	29	35
Total	2	18	97	117

[1]Feeding regimes: HB, JR = Extensive feeding regimes in Hebron and Jericho with high and low concentrate supplement, respectivelty; EZ= Intensive feeding regime in Ezerriah.
[2]κ-casein alleles: AA = κ-casein AA genotype; AB = κ-casein AB genotype; BB = κ-casein BB genotype.

The frequency of genotypes AB and BB was 0.125 and 0.875 in HB and 0.20 and 0.80 in JR, while in EZ, frequency was 0.057, 0.114 and 0.829 for AA, AB and BB, respectively (Table 3). The gene frequencies were calculated based on Mendelian inheritance and the frequencies of A and B were found to be, respectively, 0.1 and 0.9 in JR, 0.06 and 0.93 in HB and 0.11 and 0.89 in EZ.

DISCUSSION

Feeding Managements: The milk quality is influenced by its composition, which depends on many factors such as environmental and farm conditions, animal genetic and feeding management [2]. In the present study, environmental condition and feed management have an important role in controlling milk quality. Generally, goats of HB depend on grazing of low-quality pasture that leads to be mainly supplemented by a moderate concentrate feed, which may explain the higher density and concentrations of SNF, protein and lactose compared with JR and tendencies for higher levels than at EZ [17]. Supplemental concentrate increases milk yield and SNF [18], but high dietary concentrate levels can negatively affect fat concentration [19, 20, 21]. Conversely, goats in JR depend primarily on grazing of low-quality forage, with small amounts of concentrate. Furthermore, JR is characterized by desert conditions with high temperature and long dry summer. The relatively high fat concentration in milk from JR for the AB genotype may be related to the shortage in feed resources, particularly supplementary feeding and the exposure to arid and desert conditions. This is in agreement with findings of Shkolnik *et al.* [4] and Maltz and Shkolnik [5], whom showing adaptation of black Bedouin goats to desert conditions and high milk fat content resulting from water scarcity and effects of the hot environment on appetite and nutrient metabolism [22]. This may be reflected by increased mRNA transcription of fatty acid binding domains [23, 24]. These domains prolonged low energy diets and promote specific heat shock protein (HSP) response that compensates for energy loses in milk and results in production of fatty milk and with high proportions of long and unsaturated fatty acids. This is not in close alignment with the global interest of milk production where genetic selection for increased milk fat percentage leads to increased proportions of short-chain fatty acids in milk fat and decreased proportions of long-chain fatty acids [25]. Eitam *et al.* [24] reported a significant increase in short-chain and saturated fatty acids in control milk that could have arisen from increased levels of fatty acid binding protein 3 in the mammary gland [26]. However, the relatively low fat content in milk from goats in EZ may be due to the lack of available pasture and feeding indoors on moderate quality hay and supplemental concentrate.

PCR-RFLP and Sequencing: The highest number of caprine CSN3 polymorphisms discovered and sequences available in the last few years have lead to inconsistentency in nomenclature [11, 27]. To avoid any confusion, we focused on the point mutation in exon 4 of the goat k-CSN3 gene that determined two allelic variants (A and B). Both variants were investigated, rather than IEF variants and distinguished by PCR- RFLP analysis using newly designed primers. The frequency of the AA genotype in goats was found to be lower than that of BB genotype (Table 3). These results were similar with the Garganica goat breed in Italy where A allele has 0.077 frequency and in Cashmere and Derivata di Siria with 0.035 and 0.076, respectively [28]. The results further confirm that goats studied are predominantly of CSN3 B genotype. The original variant is expected to have the highest frequency over a large number of populations as shown with Caravaca *et al.* [29] which AB and BB genotypes were significantly associated with higher levels of total protein content compared with the AA *CSN3* genotype.

Feeding Management under Gene Control: The similar significant interactions ($P < 0.05$) were found in density and SNF content and its fractions, protein and lactose. These parameters were similar among feeding regimes with BB genotype but different with AB genotype in which a significant difference was noticed between HB and JR, while values obtained with EZ were intermediate. This significant interaction reflected the detected one with fat, which increasing fat content will negatively affect the SNF content. This reflected the effect of available feed resources for goats at these different areas, which is confirmed the relationship between milk composition and environmental condition reported by Sauvant and Schmidely [2] and Macdonald *et al.*[30]. However, this significant interaction showed with the SNF and its fraction indicated that the effect of environment and feeding regime on milk composition is controlled by the CSN3 gene. The effect of feeding regime on milk composition (SNF and its fractions, protein and lactose) and density was not significant associated with BB genotype. Otherwise, this effect showed significant and clear with AB genotype as mentioned above. This is an indicator to confirm that effects of feeding regime on milk composition and quality are genetically controlled by the k-casein polymorphism gene. Results are difficult to interpret because the CSN3 A and B variants only differ by a single amino acid substitution at position 471. The A allele has a valine and the B has an isoleucine [12] which both are aliphatic amino acids with similar biochemical properties. However, this may be considered as a marker for herd selection to control milk production and composition in which the AB heterozygote may serve to

alter milk composition as relating to feeding management and milk industry required. The BB genotype may serve to maintane high levels of milk production in high producer's dairy animals by increasing the proportion of concentrate feed to supplement nutritional requirements, not supplied by the forage, without negative effects on body weight, reproductive performance and milk composition and quality.

Finally, in addition to the effect of environmental conditions and feed management in controlling milk quality, the results further confirm that goats studied are predominantly of CSN3 B genotype. Moreover, it was of particular interest to give prerequisite for the effect of Single nucleotide polymorphism (SNP's) or the polymorphism of single gene on milk components and start to adopt this technology and incorporate it into dairy goat program which could be an interested breeding objective. Further studies are needed to support these results that could help in superior selection and promising option for enhancing the quality of goat milk production.

CONCLUSION

The milk composition for Palestinian goat breeds concludes that milk composition is affected by different environmental conditions, such as feeding regimes. The significant interaction between allele and different feeding regime was shown on density, SNF, protein and lactose levels. This indicated that effects of environment or feeding regime on milk composition are controlled by genetic background that could help in superior selection and promising option for enhancing the quality of goat milk in the Middle East region.

ACKNOWLEDMENT

This work was supported by the USAID Middle East Regional Cooperation (MERC) program; project number PCE-G-00-00-00026, entitled "Multinational Approaches to Enhance Goat Production in the Middle East". The authors would like to thank Dr. A. L. Goetsch from American Institute for Goat Research, Langston University, USA and Dr. H. Zaky from the Desert Research Center, Egypt, for their advice and help in supporting this work.

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