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DNA Polymorphism Studies of β-Lactoglobulin Gene in Saudi Goats

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Abstract**—**Domestic goats (*Capra hircus*) are extremely diverse species and principal animal genetic resource of the developing world. These facilitate a persistent supply of meat, milk, fibre, and skin and are considered as important revenue generators in small pastoral environments. This study aimed to fingerprint β-*LG* gene at PCR-RFLP level in native Saudi goat breeds (Ardi, Habsi and Harri) in an attempt to have a preliminary image of β-*LG* genotypic patterns in Saudi breeds as compared to other foreign breeds such as Indian and Egyptian. Also, the Phylogenetic analysis was done to investigate evolutionary trends and similarities among the caprine β-*LG* gene with that of the other domestic specie, viz. cow, buffalo and sheep. Blood samples were collected from 300 animals (100 for each breed) and genomic DNA was extracted. A fragment of the β-*LG* gene (427bp) was amplified using specific primers. Subsequent digestion with *Sac II* restriction endonuclease revealed two alleles (A and B) and three different banding patterns or genotypes i.e. AA, AB and BB. The statistical analysis showed a general trend that β-*LG* AA genotype had higher milk yield than β-*LG* AB and β-*LG* BB genotypes. Nucleotide sequencing of the selected β-*LG* fragments was done and submitted to GenBank NCBI (Accession No. KJ544248, KJ588275, KJ588276, KJ783455, KJ783456 and KJ874959). Phylogenetic analysis on the basis of nucleotide sequences of native Saudi goats indicated evolutional similarity with the GenBank reference sequences of goat, *Bubalus bubalis* and Bos *taurus*. However, the origin of sheep which is the most closely related from the evolutionary point of view, was located some distance away

Keywords**—**β-Lactoglobulin, Saudi goats, PCR-RFLP, Phylogenetic analysis.

I. Introduction

GOAT are the most versatile domestic animals in adaptation to extreme climatic conditions and desert and mountain conditions [1]-[3]. Goat milk is characterized with its offensive odor. This is especially from buck whose odor can affect the flavor of the milk. Improper or insufficient ventilation, milking practices and cooling of milk can cause this unpleasant odor of milk. Recently milked and cooled goat milk is odor free and hard to distinguish from cow milk in odor and taste [4]-[7]. There is variation regarding milk yield between and within different goat breeds. Scientists have found that milk production is subjective to genetic alterations in some genes coding for whey proteins [8], [9].

β-Lactoglobulin (β-LG) is a major whey protein in the milk of ruminants. It is also found in the milk of other mammals, but absent from the milk of rodents, lagomorphs or humans [10], [11]. Studies have revealed β-LG-pectin complexes as molecular nano-vehicles for delivering hydrophobic nutraceuticals such as ω-3 polyunsaturated fatty acids and Vitamin D [11]-[15]. Other biological activities of β-LG include enzyme regulation, the neonatal acquisition of passive immunity, a source of bioactive peptides and antimicrobial activity against mastitis-causing bacteria. It is actively employed in the food industry for numerous characteristics [11], [14].

Different β-LG variants have been identified in *Bos* genus (*B. taurus, B. javanicus* and *B. grunniens*) at the protein and DNA levels [14], [16]. Studies have also established numerous β-*LG* polymorphisms in sheep with three genetic variants (A, B, and C). Potential association between β-*LG* mutations and yield, composition, and cheese production has been widely studied in different sheep traits [17]. Preliminary investigations have reported novel SNPs in the goat β-*LG* gene [8], [18]. Exon 7 of the caprine β-*LG* gene comprises most of the 3' non-coding region on the mRNA. Thus, none of the variants described generates any amino acid change in the protein. However, it is known that the 3' non-coding region of the *m*RNA plays an important role in stabilizing the messenger, determining the half life of the molecule and hence the translation of the transcript [18].

Conservation of the local genetic resources has been acquired great importance nowadays. Saudi Arabia apparently to lack sufficient genetic information about the most of their local animal breeds, the current preliminary study tries to put focus on the phylogenetic analysis of Saudi breeds and linkage of the other foreign goat breeds, as also with other breeds such as sheep, cow, buffalo breeds. This would allow sustained genetic improvement, and to facilitate rapid adaptation to changing breeding objective [9].

Ardi, Habsi and Harri are the native Saudi Arabian goat breeds. They are essential in terms of regional livestock agriculture but lack detailed genetic characterization. Ardi and Harri goats are drastically similar (73.5%) compared to Habsi goats. Harri breed is famous for high milk yield while Ardi is known among the farmers cause of constant milk production [19], [20]. There are few details known regarding Habsi goats. The traditional breeders think of Habsi far more productive

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and our statistics also significantly support this notion. Preliminary regional studies have found novel β-*LG* novel genetic variants in goats [8], [9].

As no preliminary data is available regarding β-*LG* polymorphism in Saudi breeds, this investigation represents an initial and exploratory study concentrated on analyzing β-*LG* genotypic patterns at the DNA-RFLP level together with investigating potential associations with utility traits like milk yield. More detailed analysis is warranted using sequencing and SNP tools for accurate genetic image of the caprine β-*LG* gene belonging to Saudi goat breeds.

II. Materials and Methods

A. Experimental Animals

Blood samples (n=100 for each breed i.e. Ardi and Harri) were collected from different geographical locations and private farms located in Jeddah province and Riyadh city, while samples belonging to Habsi breed (n=100) were obtained from two farms located near Al-Qunfudhah village, South Jeddah.

B. Milk Recording and Statistical Analysis

Goats were reared under strict husbandry conditions. Milk yield was recorded 3 days per week during the first 16 weeks of lactation. Total milk yield for each studied breed was statistically analyzed by one way ANOVA at significance level ($P<0.05$). Genotypes were determined by direct counting of restriction fragments observed in the gel. Genotype frequency=No of individuals of particular genotype/Total No of individuals of all genotypes [21].

C. Blood Collection and DNA Isolation

Around, 10 ml of venous blood was collected from the jugular vein of each goat using 0.5 ml EDTA (ethylene diamine tetra acetate, 0.5 M, pH=8) as an anticoagulant. The samples were transported to the animal genetics laboratory in double walled iceboxes with ice packs and kept at -20 °C until the isolation of genomic DNA. Goat genomic DNA was extracted from the frozen blood samples via QIAamp DNA extraction kit.

D. PCR-RFLP

A region of the β-*LG* gene spanning over exon 7 to 3′ flanking area was amplified by employing a set of forward (5'CGGGAGCCTTGGCCCCTCTG3') and reverse (5'CCTTTGTCGAGTTTGGGTGT3') primers [8]. The reaction recipe contained 1 µl (10 pmol) of each primer, 0.5 µl (200 µM) dNTP mix, 0.5 µl (1.0 U) Taq DNA polymerase, 2.5 µL of 10 × High yield buffer complete with $MgCl_2$ and 2 µl (100 ng) genomic DNA template (Amplification Profile in Table I). PCR amplicons were digested overnight at 37 °C with 10 Units of *Sac II* restriction endonuclease. All the constituents requisite for enzymatic reaction were prepared at below zero temperatures. Restriction enzyme, 10 × reaction-buffer and autoclaved TDW were mixed for all the mandatory reactions in a 1.5 ml micro-centrifuge tube to design a master mix which was then dispensed into labelled 200 µl PCR tubes. Specific amplicons were added to the corresponding tubes and incubated at 37 °C for overnight reaction. Following digestion, the samples were resolved in 3% (w/v) agarose gel in 1 × TAE buffer stained with ethidium bromide for distinguishing genotypes. The gels were visualized under UV light on a trans-illuminator to detect the banding patterns and were recorded in a gel documentation system.

E. Phylogenetic Analysis

Selected β-*LG* fragments were sequenced using Sanger's di-deoxy chain termination method [22]. The Phylogenetic analysis was done to reveal evolutionary trends among different specie compared to caprine exon 7 sequence. It was achieved using MEGA V6.0 software.

TABLE I
Thermal Cycler Parameters

1	An initial cycle at 95°C for 5 min
2	35 cycles of steps containing Denaturation at 95°C for 30 s Annealing at 64°C for 1 min Extension at 72°C for 90 s
3	Final extension at 72°C at 5 min

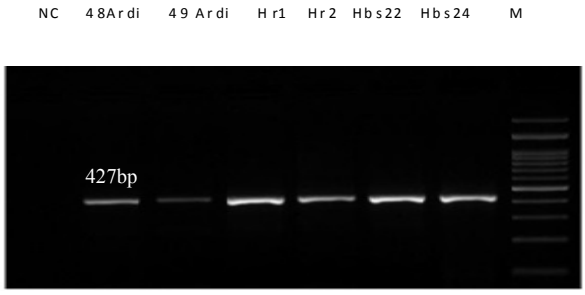

Fig. 1 Amplification of the β-*LG* from exon 7 to the 3' flanking region (Band Size: 426 bp). Lane 1st: 100 bp DNA ladder

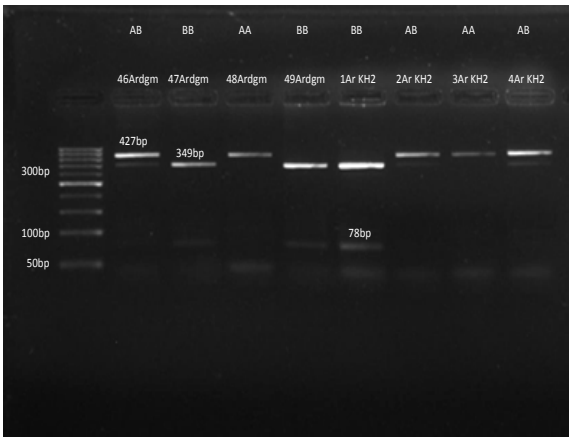

Fig. 2 PCR-RFLP electrophoretic patterns of β-*LG* amplicons digested with *Sac II* revealing different genotypes in studied goat breeds. Lane 1st: 50 bp DNA ladder

III. Results and Discussion

A. PCR-RFLP Analysis

The 427 bp β-*LG* amplicons (Fig. 1) were digested with *Sac II* restriction endonuclease to establish polymorphic sites in

exon 7 and 3' flanking region. *Sac II* revealed two alleles (A and B) with three different restriction patterns or genotypes (AA, AB and BB) (Fig. 2). The β-*LG* AB genotype had two restriction sites and generated three bands i.e. 427 bp, 349 bp and 78 bp. The β-*LG* BB genotype with only one restriction site revealed two bands of sizes 349 bp and 78 bp. An undigested product of size 427 bp termed as β-*LG* AA genotype was also obtained.

The distribution of genotypic and allelic frequencies is presented in Table II. The frequency of A allele was found to be lower compared to that of the B allele in all the studied breeds, and in close agreement to the data presented earlier by [8] in Indian goats. Given the predominance of B allele over the A allele, the B allele could possibly be taken as the ancestral variant of the β-*LG* gene in Saudi goats.

As obvious from Table II, Habsi breed with the highest presence of A allele showed significantly higher milk yield (138.26 ± 1.26 liters) than Ardi (132.11 ± 1.08 liters) and Habsi (131.32 ± 1.08 liters) breeds (P<0.05). The results presented in this study are in close agreement with the data generated by [8] in Indian goats and with the results of [9] where higher AA genotype in Damascus goat breed was associated with higher milk yield in this breed compared to Barki and their crossbred which have lower AA genotype frequency than Damascus. On the other hand, there is little information available about similar affects in sheep and cattle [23], [24].

Complete sequence of the caprine β-*LG* gene has been described [25]. One variant was found in the β-*LG* 5' flanking region (710 bp) in Chinese dairy goats [26]. The authors demonstrated that milk yield of individuals with genotype AA was higher than that with genotype AB in second and third lactation milk yield and average milk yield (P<0.05). The results implied that allele A of β-*LG* 5' flanking region is probably related to high milk protein yield. Genetic polymorphisms in the 5' flanking region (promoter region) was also noted [27]. The present study also significantly illustrated that the β-*LG* AA genotype had a higher milk yield than the β-*LG* AB and BB genotypes in native Saudi goat breeds i.e. Ardi, Habsi and Harri in relation to parity. Further studies are warranted in order to access polymorphism at the nucleotides sequence level and to get more accurate image about the molecular typing of this gene in Saudi Arabian goat breeds.

PCR- RFLP fingerprinting can be used as a preliminary tool in selecting superior genetic structures for milk production in young female goats in shorter time than the traditional selection could. The selection of these superior individuals in early age and culling of the lower ones based on their genetic structures could participate in improving milk production from local adaptive goats [9].

TABLE II
GENOTYPIC AND ALLELIC FREQUENCIES OF THE B-*LG* GENE AND ASSOCIATED MILK YIELD IN THREE DIFFERENT GOAT BREEDS FOLLOWING *SAC* II DIGESTION

		Genotypic frequency			Allelic frequency		Milk yield (Kg/16weeks)
Breed	N	AA	AB	BB	A	B	(Mean±SE)
Ardi	100	0.08	0.4	0.52	0.28	0.72	132.11±1.08[a]
Habsi	100	0.23	0.41	0.36	0.43	0.57	138.26±1.26[b]
Harri	100	0.09	0.34	0.57	0.26	0.74	131.32±1.08[a]

(a, b=P<0.05)

B. Phylogenetic Analysis

Phylogenetic analysis of Saudi breeds and linkage of the other foreign goat breeds, as also with other breeds such as sheep, cow, buffalo breeds is shown in Fig. 3. The sequence information of goat β-*LG* fragments was compared with already published β-*LG* sequences of goat (NCBI Accession No. Z33881), *Bubalus bubalis* (NCBI Assession No. JF274007), *Bos taurus* (NCBI Accession No. X14710) and *Ovis aries* (NCBI Accession No. X12817). Phylogenetic analysis on the basis of nucleotide sequences of native Saudi goats indicated similarity with the reference β-*LG* sequence of goat (NCBI Accession No. Z33881), *Bubalus bubalis* (NCBI Assession No. JF274007) and *Bos taurus* (NCBI Accession No. X14710). However, the origin of sheep (NCBI Accession No. X12817) which is the most closely related from the evolutionary point of view, was located some distance away (Fig. 3).

Fig. 3 Phylogenetic analysis based on nucleotide sequence of the β-*LG* fragments of Saudi goats (Ardi, Habsi and Harri breeds) with GenBank β-*LG* reference sequence of goat (NCBI Accession No. Z33881), *Bubalus bubalis* (NCBI Accession No. JF274007*), Bos taurus* (NCBI Accession No. X14710) and *Ovis aries* (NCBI Accession No. X12817)

As it is obvious from the current study, phylogenetic analysis of β-LG gene in Saudi goat breeds represent initial and important step for acquiring genetic information about these local breed and in the same time could contribute in conservation of these genetic resource. On the other hand, this step should be followed by other detailed study using other precious fingerprinting tools such as DNA sequencing and SNP in order to get more detailed results about the genetic variation within and between these breed and also with the other breeds. This information would be of greatest importance in the future for enhancing the productive and genetic performance of these breeds.

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REFERENCES

[1] Gall C.F. (1991): Breed differences in adaptation of goats. Pages 413-429 B8, in K Maijala, ed., genetic Resources of pig, sheep and goat, Elsevier Science publ. Amsterdam . The Netherlands, world Animal Science series.

[2] Quartermain A.R. (1991): Evaluation and utilization of goat breeds. Pages 451-470 B8, in K. Maijala, ed, Genetic Resources of pig, sheep and goat. Elsevier Science publ., Amsterdam. The Netherlands, world Animal Science Series.

[3] Silanikove N. (2000) : The physiological basis of adaptation in goats to harsh environments . Small Ruminant Research, 35, 181-193.

[4] Mowelm A. (1988): Goat farming . Farming press. Ipswich, U.K. , 183 p

[5] Zeder M.A., and Hesse B. (2000). The initial domestication of goats (Capra hircus) in the Zagros Mountains 10,000 years ago. Science, 287, 2254-2257.

[6] MacHugh D.E., and Bradley D.G. (2001): Livestock genetic origins: goats buck the trend. Proceedings of the National Academy of Sciences USA, 98, 5382-5384.

[7] Qureshi M.I., Sabir J.S.M., Mutawakil M.H.Z., El Hanafy, A.A., Ashmaoui H.E., Ramadan, H., Anwar Y., Sadek A.M., Alsoud, M.A., Saini, K.S., Ahmed, M.M. (2014). Review of modern strategies to enhance livestock genetic performance: From molecular markers to next-generation sequencing technologies in goats. Journal of Food, Agriculture & Environment, 12, 7 5 2-7 6 1.

[8] Kumar A., Rout P.K., Roy R. (2006): Polymorphism of beta-lactoglobulin gene in Indian goats and its effect on milk yield. Journal of Applied Genetics, 47, 49-53.

[9] EL-Hanafy A.A., El-Saadani M.A., Eissa M., Maharem G.M., Khalifa Z.A. (2010): Polymorphism of β-lactoglobulin gene in Barki and Damascus and their crossbred goats in relation to milk yield. Biotechnology in Animal Husbandry, 26, 1-12.

[10] Hambraeus L., Lonnerdal B. (2003): Nutritional aspects of milk proteins. In P. F. Fox, & P. L. H. M. McSweeney (Eds.), Advanced dairy chemistry (I. Proteins 3rd Ed. Part B). New York, USA: Kluwer.

[11] Kontopidis G., Holt C., Sawyer L. (2004): Invited review: beta-lactoglobulin: binding properties, structure, and function. Journal of Dairy Science, 87, 785-96.

[12] Wang Q.W., Allen J.C., Swaisgood H.E. (1997): Binding of vitamin D and cholesterol to β-lactoglobulin. Journal of Dairy Science, 80, 1054-1059.

[13] Zimet P., and Livney Y.D. (2009): Beta-lactoglobulin and its nano-complexes with pectin as vehicles for omega-3 polyunsaturated fatty acids. Food Hydrocolloids, 23, 1120-1126.

[14] Cui Y., Cao Y., Ma Y., Qu X., Dong A. (2012): Genetic variation in the B-lactoglobulin of Chinese yak (Bos grunniens). Journal of Genetics, 91, 44-8.

[15] Ron N., Zimet P., Bargarum J., Livney Y.D. (2010): Beta-lactoglobulin-polysaccharide complexes as nanovehicles for hydrophobic nutraceuticals in non fat foods and clear beverages. International Dairy Journal, 20, 686-693.

[16] Caroli A.M., Chessa S., Erhardt G.J. (2009): Invited review, milk protein polymorphisms in cattle, effect on animal breeding and human nutrition. Journal of Dairy Science, 92, 5335-5352.

[17] Arora R., Bhatia S., Mishra B.P., Sharma R., Pandey A.K., Prakash B., Jain A. (2010): Genetic polymorphism of the beta-lactoglobulin gene in native sheep from India. Biochemical Genetics, 48, 304-11.

[18] Pena R.N., Sanchez A., Folch J.M. (2000): Characterization of genetic polymorphism in the goat beta-lactoglobulin gene. Journal of Dairy Research, 67, 217-224.

[19] Sabir J.S.M., Mutawakil M.H.Z., El-Hanafy A.A., Ahmed M.M.M. (2012): Genetic similarity among four breeds of goats in Saudi Arabia detected by random amplified polymorphic DNA marker. African Journal of Biotechnology, 11, 3958-3963.

[20] Sabir J.S.M., Sabry A.M., Awad N.S., Mohamed A.A., Mutawakil M.H.Z. (2013): Najdi, Harri and Aradi Saudi Goat Breeds Possess Genetic Variation Required for Genetic Improvement. World Applied Sciences Journal, 26, 867-872.

[21] Falconer D.S., Mackay T.F.C. (1996): Introduction to Quantitative Genetics. 4th Ed. Addison Wesley Longman, Harlow, Essex, UK.

[22] Sanger F., Nicklen S., Coulson A.D. (1977): DNA sequencing with chain terminating inhibitor. Proceedings of the National Academy of Sciences USA, 74, 5436-5467.

[23] Kawecka A., Radko A. (2011): Genetic polymorphism of β-lactoglobulin in sheep raised for milk production. Journal of Applied Animal Research, 39, 68-71.

[24] Botaro B.G., Lima Y.V., Aquino A.A., Fernandes R.H., Garcia J.F., Santos M.V. (2008): Effect of beta-lactoglobulin polymorphism and seasonality on bovine milk composition. Journal of Dairy Research, 75, 176-181.

[25] Folch, J.M., Coll, A. And Sa´nchez, A. (1994). Complete sequence of the caprine β-lactoglobulin gene. Journal of Dairy Science 77: 3493-3497.

[26] Chen, H., Lan, X.Y., Li, R.B., Lei, C.Z., Sun, W.B., Zhang, R.F., Zheng, Y.L., Zhu, B.C. (2005). The effect of CSN1 S2, CSN3 and beta-lg genes on milk performance in Xinong Saanen dairy goat. Yi Chuan Xue Bao 32 (8): 804-810.

[27] Yahyaoui, M.H., Angiolillo, A., Pilla, F., Sanchez, A., Folch, J.M. (2003b). Characterization and genotyping of the caprine kappa casein variants. Journal of Dairy Science 86: 2715-2720.