Livestock Science 196 (2017) 49–54

Contents lists available at ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

Short communication

Single nucleotide polymorphism of candidate genes in non-descript local goats of Sri Lanka

H.B.P.C. Ariyarathne, H.B.S. Ariyaratne, L.G.S. Lokugalappatti*

Department of Basic Veterinary Sciences, Faculty of Veterinary Medicine and Animal Science, University of Peradeniya, Peradeniya 20400, Sri Lanka

ARTICLE INFO

Keywords:
Non-descript local goats
k-CSN3
LALBA
GnRHR
SNPs
DNA sequencing

ABSTRACT

In the present study, genetic polymorphism in exon 4 of kappa casein (*k-CSN3*), exon 2–3 of alpha lactalbumin (*LALBA*) and exon 1 of gonadotropin releasing hormone receptor (*GnRHR*) genes were analyzed as candidate genes for milk production, milk quality and prolificacy aiming to provide information for future studies on genetic improvements in non-descriptive local goats in Sri Lanka. Altogether eleven, thirteen and three single nucleotide polymorphisms (SNPs) were identified in *k-CSN3, LALBA* and *GnRHR* gene fragments respectively utilizing the DNA sequencing technique in Sri Lanka. Seven polymorphic sites out of eleven in *k-CSN3* gene fragment and the recorded polymorphic site in exon 3 of *LALBA* gene fragment were homozygous while all three polymorphic sites in *GnRHR* gene fragment were heterozygous. Two of the SNPs recorded in the present study are found to unique for Sri Lankan non-descript goat population at G203T and A730G in *k-CSN3* and *GnRHR* genes respectively. The study records another two SNPs in *GnRHR* gene which are already known to be correlated with higher fecundity in goats (G757A and G891T). Results of the present study will be extremely important in future attempts to indicate markers to improve the milk production, composition of milk and litter size of non-descript local goats in Sri Lanka.

1. Introduction

Sri Lankan non-descript local goats are highly localized, heterogeneous group which covers the majority of the goat population in the country (National Livestock Breeding Policy, 2010) and are currently known to be under the threat of extinction due to intensive cross breeding with exotic breeds (Silva, 2010). These non-descript goats are much more important at local circumstances as they hold invaluable genetic resources for high adaptability to local environmental conditions, disease resistance (Silva, 2010) and high degree of prolificacy (National Livestock Breeding Policy, 2010) though, they are poor in production parameters compared to exotic breeds (Silva, 2010). Low performance in production of non-descript goats may be the result of poor management practices in rural areas and genetic makeup of animals (Azevedo et al., 1994). However, SNPs in production related genes affect significantly on the phenotypic characters though they arise at a lower rate in a population (Cargill et al., 1999) and production parameters can be greatly enhanced at a minor cost by promoting the inheritance of these beneficial mutations to next generation via selection. Consequently, modern researches are at an inspired interest on association analysis of SNPs and related phenotypic characters such as milk, meat and wool production and prolificacy traits. *k-CSN3* and *LALBA* genes are milk trait related and *GnRHR* is a fecundity trait related gene which are collectively widely studied genes for genetic variability. Many researchers have reported the association between *k-CSN3* genetic variability and milk composition (Chiatti et al., 2005; Marletta et al., 2007) and milk production (Marletta et al., 2007). Further studies have revealed the association between *LALBA* gene polymorphism and nutrient content of milk (Lan, 2007, An, 2009, Zidi, 2014) and relationship between genetic variability of *GnRHR* gene and prolificacy trait (Ming-Xing et al., 2009, Yang et al., 2011). The inclusive goal of this study was to identify SNPs in Caprine *k-CSN3*, *LALBA* and *GnRHR* genes aiming to genetically characterize non-descript local goats of Sri Lanka, thereby to offer genetic clues for future association analysis.

2. Material and methods

2.1. Collection of samples

A sample of 112 non-descript local goats from Eastern (49), Northern (15), Northwestern (37), and Southern (11) provinces was used for the study to cover different localities of Sri Lanka. Blood was collected from animals into sterile vacutainer tubes containing 100 µg/

* Corresponding author.
E-mail address: slokug@pdn.ac.lk (L.G.S. Lokugalappatti).

http://dx.doi.org/10.1016/j.livsci.2016.12.012
Received 1 September 2016; Received in revised form 28 November 2016; Accepted 23 December 2016

ml Ethylene diammine tetraacetic Acid (EDTA) using jugular puncture method.

2.2. Extraction, PCR amplification and Sequencing of DNA

DNA extraction from collected blood samples was done using salting-out protocol (Jawdat et al., 2011) and commercially available kits (Wizard Genomic DNA Purification Kit). Extracted genomic DNA was visualized on Ethidium bromide stained 1% agarose gels and quantified using a DNA spectrophotometer (BIOMATE 3). Exon 2–3 of Caprine *LALBA* (part of exon 2, intron 2 and part of exon 3) (667 bp), exon 4 of *k-CSN3* (458 bp) and exon 1 of *GnRHR* (746 bp) gene fragments were amplified using already published protocols by Jain et al., 2009; Kiplagat et al., 2010 and Yang et al., 2011 respectively. PCR was performed in a 25 µl reaction mixture containing 50 ng genomic DNA, 0.5 µM of each primer, 1× PCR reaction buffer, 1.5 mM of MgCl2, 200 µM of dNTPs and 0.625 units of *Taq* DNA polymerase and amplifications were carried out in an ABI2720 (Applied Biosystems®) Thermal Cycler. Amplified PCR products were then visualized on 1% Agarose gel stained with Ethidium Bromide (1 µg/ml) and verified using a 100 bp ladder (Vivantis 6x). Following the visualization, PCR band was excised out of the agarose gel and PCR product was purified using PureLink PCR gel clean-up kit (Qiagen) and quantified using Lambda markers. All 112 samples were sequenced for screening genetic variations using Bigdye terminator chemistry in an ABI 3500 genetic analyzer.

2.3. Sequence alignment

All DNA sequences were checked against Caprine genome sequences (BLAST+2.3.0) already published in Genbank and for presence of any insertions, deletions and for absence of stop codons using Sequencher software v 5.0.1 (Gene Codes Corp). Bases ambiguities were visualized and manually edited using CodonCode Aligner 5.1.5 (Codon Code Corporation, Dedham, MA, USA) and heterozygous bases were separated with the aid of PHASE 2.1 (Stephens et al., 2001). Poor allelic phase estimates (below Phred20 score) revealed by PHASE 2.1 were excluded from the analysis.

2.4. Polymorphic site mining

All the aligned sequences for all three gene fragments were realigned with corresponding gene sequences of other Caprine breeds already published in the GenBank database (accession no: L42937.1for *GnRHR*; X60763 for *k-CSN3* and M63868 for *LALBA* genes) and polymorphic sites were visualized using Sequencher (v 5.3). Haplotypes list was identified using PHASE 2.1 and their frequencies were calculated using Hardy-Weinberg low with the aid of MS Excel 2010. DNA sequences were translated to amino acid sequences using ExPASy translate tool (Gasteiger et al., 2003).

3. Results and discussion

Three polymorphic sites were identified from *GnRHR* exon 1 region of non-descript local goats. All three polymorphic sites were heterozygous (Fig. 1) and two of the sites were represented by G/A and the other was G/T alleles (Table 1). Heterozygous genotypes found at the polymorphic sites were represented by a minor population of goats while the majority of animals were represented by homozygous genotype. At both G757A and G891T loci G was the predominant allele whereas at locus A730G, A was predominant. The most common haplotype AA, GG, GG was represented by 17 goats out of 31 (57%). There were two haplotypes (AA, A/G, G/T and A/G, GG, GG) with similar frequency of occurrence and represented the rarest recombination (3%). Six possible allelic recombinations were predicted and AGG (74%) and AAT (1%) were among the highest and lowest expected haplotypic combinations respectively. From the six predicted haplotypes, five haplotypes (GenBank accession number: KX943532-KX943536) were carried by the goats analyzed in this study and only AAT combination was not recorded. Commonly recorded haplotype was AGG (69%) whereas GGT (2%) was the rarest among non-descript goats studied (Table 1). Analysis of the deduced amino acid sequences in A730G and G757A sites showed presence of silent mutations and reported G891T variation is non-synonymous (Table 2). Studies by Yang et al., (2011) have reported the same two SNPs at G757A and G891T. To date, the reported synonymous mutation at A730G was not recorded from any goat breed of the world. Boer goats with GG and GT genotypes at G757A and G891T positions respectively showed a significantly positive correlation with the litter size ($p < 0.05$) (Yang et al., 2011). The present study was aligned with the findings of Yang et al., 2011 by demonstrating the genotype GG (at G891T and G757A). Even a silent mutation can have positive effects on prolificacy by changing the density of *GnRHR* on the gonadotropes as a result of increased or decreased concentrations of LH and FSH (Schubert et al., 2000).

Altogether 13 polymorphic sites were detected from exon 4 and partial intervening region of *k-CSN3* gene of the studied local goat population. Eleven out of thirteen polymorphic sites were located in exon 4 and two of them were located in intron 4 region (G656A and G657A) hence, are not responsible for amino acid variations of the corresponding peptide chain. Among all the polymorphic sites in exon 4, only 4 of the sites were heterozygous (Fig. 2) while 7 other sites were homozygous (Table 2). At all four heterozygous sites the dominant genotype was homozygous and minority of the population was represented by the heterozygous genotype. At A274G and G309A polymorphic sites, all three genotypes (GG, AA, AG) were present and G was the dominant allele (0.525 and 0.9 respectively). At A471G and T591C sites only two genotypes were existed and allele A and T were prominent at 0.9 and 0.925 respectively where homozygous recessive genotypes were absent. Genotype GG at A471G and genotype CC at T591C were not recorded. At heterozygous polymorphic sites nine haplotypes were recorded. The most frequent haplotype at heterozygous polymorphic sites was GG/ GG/ AA/ TT (35%) and represented by 7 animals and was followed by AG/ GG/ AA/ TT (15%) and AA/ GG/ AA / TT (15%) haplotypes. Altogether those three genotypes cover 65% of the variability in the whole population and the remaining 35% split among other six haplotypes. Nine haplotypic combinations (GenBank accession number: KY003218-KY003226) out of ten predicted allelic haplotypic combinations were present in the studied population of goats. Among them GGAT was the dominant haplotype (23%) while GAAT (1%) and GGAC (1%) were the rarest and GAAC combination was not reported (Table 2). Analysis of the deduced amino acid sequences showed point mutations at each polymorphic site and all of them were accountable as non-synonymous variations except for the site T245C and A284G (Table 3). However, to date the nucleotide substitution at 203 site (responsible for transition of G to T and Gln to His in nucleotide and amino acid sequences respectively) has not been reported from any goat breed of the world and might be another unique polymorphic site for Sri Lankan indigenous goat population (Table 3). Previous studies have revealed the presence of 16 polymorphic sites within complete exon 4 of *k-CSN3* gene of domestic goats (Prinzenberg et al., 2005). Thirteen out of 16 polymorphic sites were responsible for protein variants and 3 were synonymous mutations (Prinzenberg et al., 2005). From the recorded 11 sites in exon 4, 10 were previously reported by many researches (Angiolillo et al., 2002; Caroli et al., 2001; Jann et al., 2004; Prinzenberg et al., 2005; Yahyaoui, 2003; Yahyaoui et al., 2001) except the novel mutation at G203T.

Many researches (Caroli et al., 2001; Prinzenberg et al., 2005; Yahyaoui, 2003; Yahyaoui et al., 2001) reported the silent mutation at 254 site. Synonymous mutation at 284 site was previously described in Spanish and French Saanen breeds (Yahyaoui et al., 2001) and in seven European breeds (Prinzenberg et al., 2005). Nucleotide A instead of G