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SEQUENCING, POLYMORPHISM AND PHYLOGENETIC CHARACTERISATION OF KISS-1 GENE IN TWO NIGERIAN INDIGENOUS GOAT BREEDS

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Abstract: KiSS-1 gene encodes a protein product kisspeptin which are intense inducers of luteinizing hormone (LH) and follicle-stimulating hormone (FSH) secretion in various mammalian species through its receptor GPR54 (G protein-coupled receptor-54). A total of 100 goat compromising of Red Sokoto (n = 72) and Sahel (n = 28) breeds were used to detect single nucleotide polymorphisms (SNPs) in the intronic region of the *KiSS-1* gene by sequencing and investigate their relationship with other goat breeds. Nucleotide sequence analysis revealed five novel SNPs (g.1745G>A present in Red Sokoto, g.1776G>A, g.1827A>G, g.1857T>C and g.2208T>C present in Red Sokoto and Sahel breeds). To obtain a correct phylogenetic relationship between goat breeds, nucleotide sequences were compared to other sequences in NCBI database using a BLASTn algorithm and retrieved for further analysis. Neighbour-joining phylogenetic relationship tree constructed revealed two distinct clusters with ancestral lineage of 100% identity. Nigerian goat breeds (Red Sokoto and Sahel) clustered into a clade with Indian goat breeds (Ganjam and Osmanabadi) while the second cluster involved eight other goat breeds. Genetic distance estimate revealed high genetic similarity between Red Sokoto and Sahel breeds as observed in their genetic distance value of 0.003. The nucleotide sequences of the two Nigerian goat breeds (Red Sokoto and Sahel) for *KiSS-1* gene were submitted to GenBank database and have accession numbers: MN122316 and MN122317, respectively. The analysis of polymorphism in *KiSS-1* gene indicates that genetic variation exists in the goat breeds studied. Therefore, attempts can be made to investigate the association of these polymorphism with reproductive traits in Nigerian goat breeds.

Keywords: Goat, Litter size, SNP, Genetic identity

Introduction

Goats spread all over the world because of their ability to adapt to varying environmental conditions and different regimes under which they are subsequently maintained (*Assan, 2014*) with over 300 distinct breeds to available for different purposes (*Hirst, 2018*). In Nigeria, goats constitute the largest group of small ruminant livestock totaling about 73.8 million (*FAOSTAT, 2016)*. Red Sokoto and Sahel goat breeds are two well adapted and predominantly found in the Northern part of Nigeria where they are majorly managed by traditional production system with an average flock size of 3-5 goats per small holder farmers constituting a good source of protein (*Makun et al., 2006*). The Red Sokoto (RS) goat is a highly prolific breed with high incidence of multiple births and twinning rate of 54% and 43% respectively (*Akpa et al., 2010*). However, the Sahel are known to have a short fine hair with different coat colours from plain white, grey, pied, dappled, black or brown goat is a multipurpose goat breed mostly reared for meat and skin production (*Adebambo, 2012*) although reproductive potentials of this breeds have not been fully explored. Both breeds have interestingly unique adaptive traits making them strive in the hot savanna such as long leggedness, long distance walking ability, feeding behavior, heat tolerance and remarkable recovery capacity from scarcity of feed resources (*Muema et al., 2009)*. With the increasing population and demand always exceeding supply for chevon (*Okewu and Iheanacho, 2015*), it is necessary to exploit ways of increasing animal protein availability other than the traditional breeding method which is by evaluating genetic variation in genes relatively affecting prolificacy in goat. Genetic markers like single nucleotide polymorphisms of some genes have been reported to be significantly associated with litter size in goats such as *POU class 1 homebox 1 (POU1F1)* gene (*Feng et al., 2011*), *KiSS-1* gene (*Cao et al., 2010; An et al., 2013a,b; Othman et al., 2015; Mekuriaw et al., 2017*) *Gonadotropin-releasing hormone receptor (GnRHR)* gene (*Yang et al., 2011; Huang et al., 2012; Bemji et al., 2018*), *KIT ligand* gene (*An et al., 2011; 2015; 2016*), *Inhibin alpha* (INHA) gene (*An et al., 2012; Sharma et al., 2015; Isa et al., 2017*). Although characterization of genetic variation in Nigerian goat breeds have been carried out using genetic markers like Microsatellite, Mitochondrial, Biochemical and SNPs (*Shoyombo et al., 2015; Awotunde et al., 2015; Ojo et al., 2017; Bemji et al., 2018; Isa et al., 2019*), there has been no published information on *KiSS-1* gene variant of Nigerian goat breeds. Therefore, this study was intended to identify single nucleotide polymorphism, genetic distance and phylogenetic relationship among two Nigerian Northern goat breeds.

Materials and Methods

Animal sampling and DNA isolation

A total of 100 goat belonging to two Nigerian indigenous breeds respectively managed semi-intensively at National Animal Production Research Institute (NAPRI), Shika-Zaria were included in this study. About 5 ml of blood sample was collected from 72 Red Sokoto goats and 28 Sahel via jugular venipuncture into vacutainer tubes containing ethylene diamine tetra acetic acid (EDTA) anticoagulant. Genomic DNA extraction was isolated from whole blood samples using Zymo Research quick-gDNA^{TM} Miniprep kit adhering to manufacturer's protocol. The concentration of DNA was evaluated by the Nanodrop spectrophotometer (ND1000; NanoDrop Technologies, USA) while the quality was virtually assessed by agarose gel electrophoresis.

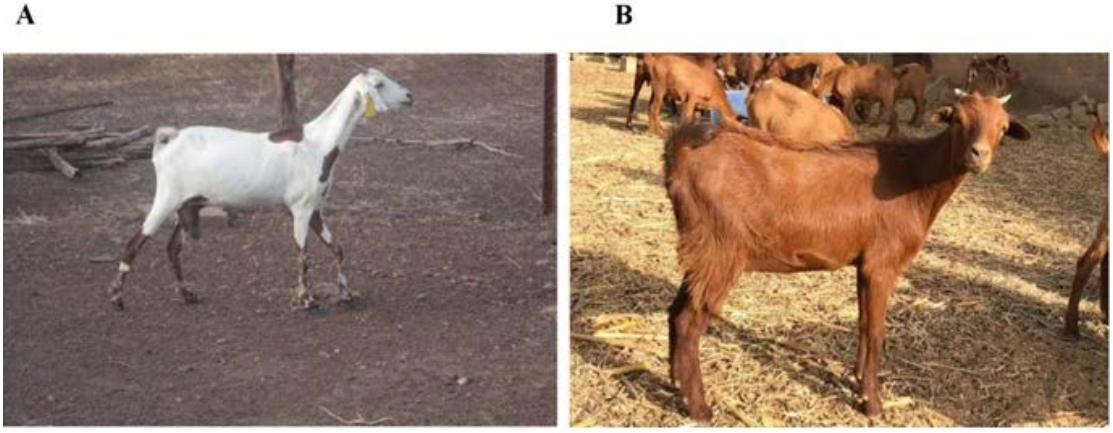

Figure 1. Pictures of two Nigerian goat breeds studied. (A) Sahel and (B) Red Sokoto

Primer design and PCR amplification

Using the caprine *KiSS-1* gene sequence (Accession number: NC_030823.1 position 1343186-1340188) a pair of primer (Table 1) was designed to amplify and sequence a 1088 bp fragment of intron 1 in Stab Vida genetics laboratory, Portugal. Polymerase chain reaction (PCR) amplification was carried out in SureCycler 8800 Thermal Cycler (Agilent Technologies, USA) with a total reaction volume of 20µL containing 50ng genomic DNA was added to a reaction mix containing 12.8µL of H2O MQ, 2.5µL of 1XPCR reaction buffer, 1µL dNTP's, 1.5µL MgCl2, pH 9, 1µL each of forward and reverse primers and 0.2 µL of *Taq* DNA polymerase. PCR

cycling protocol was accomplished by an initial denaturation at 96°C for 15 minutes followed by 40 cycles of denaturing at 95°C for 30 seconds, annealing at 58°C for 30 seconds, extension at 70°C for 1 minute and 40 seconds and final extension at 70°C for 5 minutes. The PCR products were subjected to 1% agarose gel stained with GelRed™ nucleic acid stain in a Thermo EC Midicell Primo EC-330 gel system containing 1XTBE electrophoresis buffer, scored using a standard 100 bp molecular ladder and viewed under UV light and photographed using a Vilber lourmat gel documentation system.

Table 1. Primer sequences, lengths, gene region, annealing temperatures and product size

Primer	Sequence (5' – 3')	Length	Gene region	PCR size	Tm (°C)
KISS-1F	CTTTCTGGGTAAGGGAGG	18	Intron 1	1088bp	57°C
KISS-1R	AGAGAGAGGCTTTGGACC	18			

F= Forward primer, R= Reverse primer

DNA sequencing and sequence analysis

The PCR products were purified from the gel using a Carboxylate Magnetic Beads technology (MCLab, USA) and eventual sequencing was done with BigDye® terminator cycle sequencing kit on the ABI 3730XI (Applied Biosystems, USA). The sequences were viewed and edited using Bioedit software *(Hall, 1999*). Multiple sequence alignment of the sequences were performed using ClustaW in MEGA-X software package (*Kumar et al., 2018*). Identification of single nucleotide polymorphism in *KiSS-1* gene was carried out using codon code aligner (Codon code Corporation Dedham, MA, USA) and MEGA-X software packages. A BLAST algorithm (*Altschul et al., 1990*) search was carried out to retrieve homologous nucleotide sequences from the NCBI GenBank (http://www.ncbi.nlm.nih.gov/BLAST) database. Nucleotide sequence showing 97-100 % similarity for *KiSS-1* gene of two Nigerian goat breeds (MN122316 and MN122317), ten goat published sequences viz. San Clemente (NC_030823.1), Barki (KP835800.1), Zaraibi (KP835799.1), Xinong Saanen (JQ806381.1), Guanzhong (JQ806382.1), Ganjam (KJ425411.1), Osmanabadi (KJ425412.1), Gaddi (MH397145.1), Lezhi Black (KR065750.1), Jining Grey (GU142847.1), sheep (Barki = KP835798.1) and a Japanese medaka fish as an out-group (NC_019863.2) were assembled. Genetic distance and phylogenetic analysis of the *KiSS-1* gene were carried out for Nigerian goat breeds, other goat breeds and fish sequences applying p-distance model. A neighbor joining tree method was used to infer phylogenetic relationship between the populations following alignment of the sequence of Nigerian goat breeds and the published sequences. The reliability of the phylogenetic tree branching was estimated using a bootstrap confidence level of 1000 replications.

Results and Discussion

Identification of single nucleotide polymorphism in *KiSS-1* gene of Nigerian indigenous goat breeds

The sequence result analysis of this study among two Nigerian indigenous goat breeds revealed novel polymorphism of *KiSS-1* intron 1 gene. Five single nucleotide polymorphisms (g.1745G>A, g.1776G>A, g.1827A>G, g.1857T>C, g.2208T>C) was identified within Intron 1 of goat *KiSS-1* gene. The breed, SNPs type and the position along the reference sequence and chromosome position are presented in Table 2. Several similar studies on *KiSS-1* (intron 1) gene have identified several polymorphism in different goat populations (*Cao et al., 2010; An et al., 2013b; Maitra et al., 2014a; El-Tarabany et al., 2017*). Although these studies are focused on the non-coding regions of the gene it has been proven that mutations in intragenic (intron) regions may be involved in alternative splicing/regulation, transcript processing, gene expression and protein function, chromosomal rearrangement (*Guey-Shin and Thomas, 2007; Sjakste et al., 2011*) of varying phenotypes and have been found to affect economic traits (*Jiang et al., 2010; Ibeagha-Awemu et al., 2014*).

Table 2. Polymorphisms identified in Goat *KiSS-1* gene and their position on reference sequence

Region	SNP identified	Type of mutation	SNPs position on reference sequence (NC_ 030823.1) bp	SNPs position on chromosome 16 (bp)	Breed where identified
Intron 1	g.1745G>A	Transition	1745bp	1341442	Red Sokoto
	g.1776G>A	Transition	1776bp	1341411	Red Sokoto and Sahel
	g.1827A>G	Transition	1827bp	1341360	Red Sokoto and Sahel
	g.1857T>C	Transition	1857bp	1341330	Red Sokoto and Sahel
	g.2208T>C	Transition	2208bp	1340979	Red Sokoto and Sahel

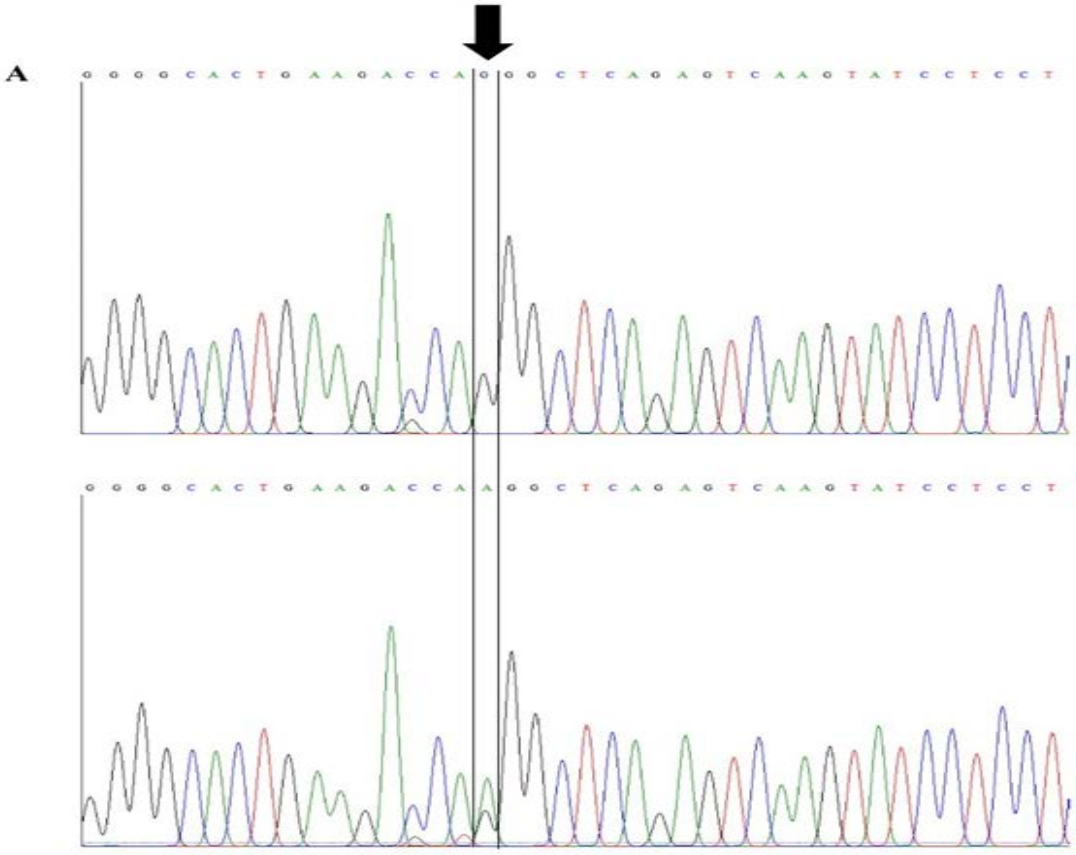

Figure 2. Single nucleotide polymorphism (SNP) variant of KiSS-1 gene in Nigerian goat breeds. (A) g.1745G>A

Genetic distance between Red Sokoto, Sahel and other goat breeds

The pair-wise estimation of genetic distance between Red Sokoto, Sahel and other goat breeds was based on sequence analysis of *KiSS-1* gene intron 1 and shown in Table 3. Among the twelve breeds sequences used for this analysis Guanzhong and Xinong Saanen had 100 percent nucleotide identity having the closest value of 0.000 and the highest was observed at 0.574 between Red Sokoto and Guanzhong, Xinong Saanen, Lehzi black. Based on the polymorphisms earlier reported in this study we observed a low pair-wise distance of 0.003 between Red Sokoto and Sahel goat breeds which compares favourable with findings of other studies estimate for Red Sokoto and Sahel (*Murital et al., 2015; Udeh, 2015; Ajibike et al., 2016; Ajayi et al., 2016; Ojo et al., 2017*). The low genetic distance estimates obtained between Red Sokoto and Sahel from different molecular markers could be attributed to migration, high degree of miscegenation or high gene flow,

geographical adaptation. Similarly, low genetic distance observed in this study would be due to shared alleles, origin and geographical adaptation between the two breeds.

Table 3. Estimated Nei genetic distance between RS, SH and other goat breeds using MEGA–X program. The standard genetic distances are below the diagonal and corrected distances are above the diagonal

	BK	ZR	XS	GZ	GJ	OS	GD	LB	RS	SH	SC	JG
BK		0.003	0.005	0.005	0.024	0.024	0.004	0.005	0.024	0.024	0.005	0.005
ZR	0.003		0.005	0.005	0.024	0.024	0.005	0.005	0.024	0.024	0.004	0.004
XS	0.011	0.013		0.000	0.024	0.024	0.004	0.003	0.024	0.024	0.004	0.005
GZ	0.011	0.013	0.000		0.024	0.024	0.004	0.003	0.024	0.024	0.004	0.005
GJ	0.564	0.564	0.569	0.569		0.005	0.024	0.024	0.003	0.003	0.024	0.024
OS	0.564	0.564	0.569	0.569	0.008		0.024	0.024	0.005	0.004	0.024	0.024
GD	0.008	0.011	0.008	0.008	0.566	0.566		0.005	0.024	0.024	0.004	0.004
LB	0.013	0.011	0.003	0.003	0.569	0.569	0.011		0.024	0.024	0.003	0.005
RS	0.569	0.569	0.574	0.574	0.005	0.008	0.572	0.574		0.003	0.024	0.024
SH	0.566	0.566	0.572	0.572	0.003	0.005	0.569	0.572	0.003		0.024	0.024
SC	0.011	0.008	0.005	0.005	0.566	0.566	0.008	0.003	0.572	0.569		0.004
JG	0.011	0.008	0.011	0.011	0.566	0.566	0.008	0.008	0.572	0.569	0.005	

BK – Barki goat breed, ZR – Zaraibi goat breed, XS – Xinong Saanen goat breed, GZ – Guanzhong goat breed, GJ – Ganjam goat breed, OS – Osmanabadi goat breed, GD – Gaddi goat breed, LB – Lezhi black goat breed, RS – Red Sokoto goat breed, SH – Sahel goat breed, SC – San clemente goat breed, JG – Jining grey goat breed

Phylogenetic relationship

The Neighbor-joining phylogenetic tree used to infer relationship between Red Sokoto, Sahel goats, some selected goat breeds and other species (*Ovis aries, Oryzias lapites*) shown in Figure 3 revealed three major clusters for which the small ruminant population had separate common ancestors at 100% reliability. First cluster estimate comprised eight exotic goat breeds and an ovine species, second cluster comprised Red Sokoto, Ganjam, Osmanabadi and Sahel, goat breeds which is indicative of these breeds having common alleles being shared and could reflect a common origin. The third clade comprised the Japanese rice (medaka) fish which from the tree had similar nucleotide sequence but branched separately showing that it is the most genetically divergent.

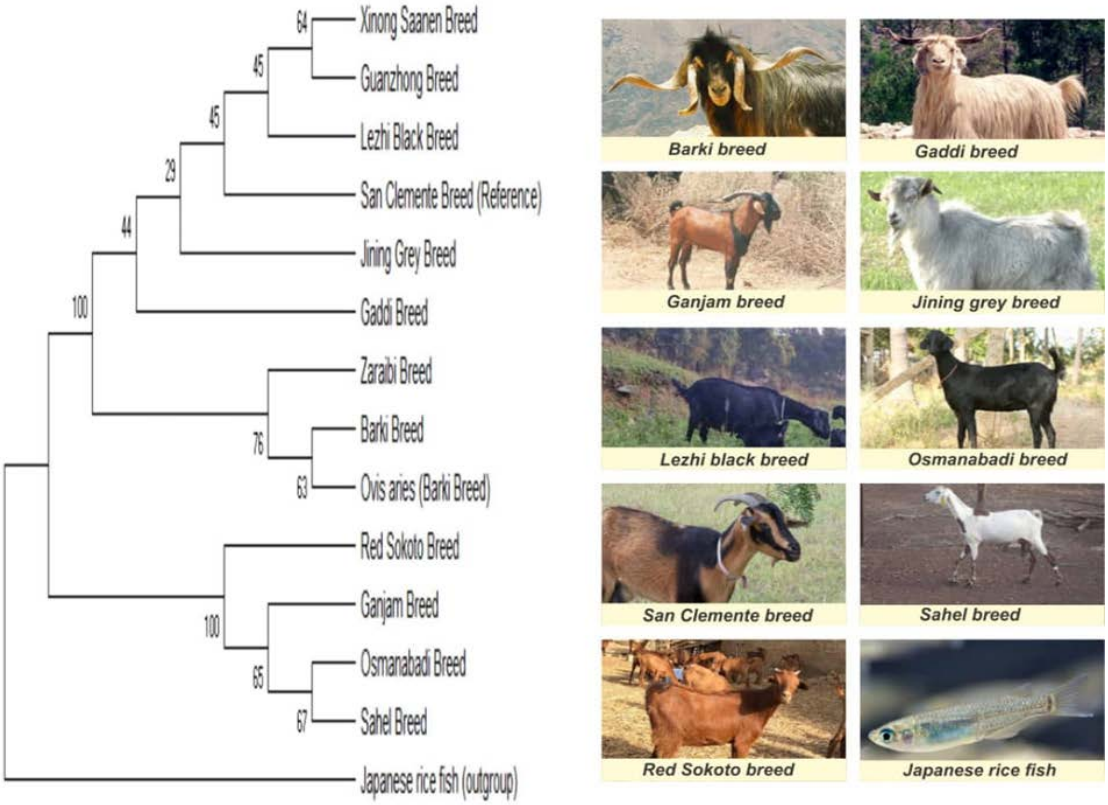

Figure 3. Neighbour-Joining phylogenetic tree between Nigerian goat breeds, other goat populations and species. Confidence bootstrap percentage values are indicated on the nodes after 1000 replications.

Conclusion

In this study, the analysis of *KiSS-1* gene of Nigerian goat breeds showed to be polymorphic. Phylogenetic relationship analysis revealed that Nigerian goat breeds (Red Sokoto and Sahel) clustered into a clade with low genetic distance (0.003) for *KiSS-1* (Intron 1) gene. The presence of genetic variation permits for genetic conservation and improvement programme of these breeds having better understanding on their genome architecture, breed relationship and phenotypic performance. It is recommended that the number of animals used in this study be increased in further studies in other to estimate genetic diversity indices and

explore the significant association of KiSS-1 gene polymorphism with litter size in Nigerian indigenous goat breeds.

Sekvenciranje, polimorfizam i filogenetska karakterizacija gena kiss-1 u dve nigerijske autohtone rase koza

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Rezime

Gen *KiSS-1* kodira proteinski proizvod kispeptin koji je intenzivni induktor lučenja luteinizirajućeg hormona (LH) i folikul-stimulišućeg hormona (FSH) kod različitih vrsta sisara putem svog receptora GPR54 (receptor vezan za G protein-54). Ukupno 100 koza rasa crveni sokoto (n = 72) i sahel (n = 28) korišćene su za otkrivanje polimorfizama jednostrukih nukleotida (SNP) u introničnom regionu gena *KiSS-1* sekvenciranjem i istraživanjem njihove veze sa drugim rasama koza. Analiza nukleotidne sekvence otkrila je pet novih SNP-ova (g.1745G>A prisutan u crvenom sokotu, g.1776G>A, g.1827A>G, g.1857T>C i g.2208T>C prisutni u rasama crveni sokoto i sahel). Da bi se dobio tačan filogenetski odnos između rasa koza, nukleotidne sekvence su upoređene sa drugim sekvencama u bazi podataka NCBI korišćenjem BLASTn algoritma i preuzete za dalju analizu. Izgrađeno stablo filogenetskih odnosa koje se spajaju sa susedima otkrilo je dva različita klastera sa 100% identitetom loze predaka. Nigerijske rase koza (crveni sokoto i sahel) udružile su se sa indijskim rasama koza (ganjam i osmanabadi), dok je u drugom klasteru učestvovalo osam drugih rasa koza. Procena genetičke udaljenosti otkrila je veliku genetsku sličnost između rasa crveni sokoto i sahel, primećeno u njihovoj vrednosti genetske udaljenosti od 0,003. Nukleotidne sekvence dve nigerijske rase koza (crveni sokoto i sahel) za gen *KiSS-1* predate su u bazu podataka GenBank i imaju pristupne brojeve: MN122316, odnosno MN122317. Analiza polimorfizma u genu *KiSS-1* ukazuje na to da postoje genetske varijacije u proučavanim rasama koza. Stoga se mogu pokušati istražiti povezanost ovog polimorfizma sa reproduktivnim osobinama kod nigerijskih rasa koza.

Ključne reči: koza, veličina legla, SNP, genetski identitet

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