Archivos de Zootecnia
Journal website: https://www.uco.es/ucopress/az/index.php/az/

Association between Myostatin gene exons 1 and 3 polymorphisms and morphological traits in Nigerian Red Sokoto goat breed

Sanni, M.T.[1@]; Okpeku, M.[2,3]; Adeleke, M.A.[4]; Wheto, M.[1]; Olowofeso, O.[1]; Oluwatosin, B.O.[5,6]; Adebambo, O.A.[1] and Ikeobi, C.O.N.[1]

[1]Department of Animal Breeding and Genetics. Federal University of Agriculture. Abeokuta. Nigeria.
[2]State Key Laboratory for Genetic Resources and Evolution. Chinese Academy of Sciences. Kunming Institute of Zoology. Yunnan Province. China.
[3]Animal Breeding and Genetics Group. Department of Animal Science. University of Swaziland. Swaziland.
[4]Disciple of Genetics. School of Life Sciences. University of KwaZulu-Natal (Westville Campus). Durban. South Africa.
[5]Institute of Food Security. Environmental Resources and Agricultural Research. Abeokuta. Nigeria.
[6]Department of Animal Production and Health. Federal University of Agriculture. Abeokuta. Nigeria.

Additional keywords
Myostatin gene.
Polymorphisms.
Morphological traits.
Red Sokoto goat.
Nigeria.

SUMMARY

Exons of genes control the genetic information that is transcribed and translated to the protein product that produces, directly or indirectly, observed phenotypes of traits. The need for the availability of genetic information, in relation to those loci which affect performance traits may be important tools in breeding programme. The research focused on the genetic, amplification and sequencing of myostatin gene from the blood of Red Sokoto goat breed of Nigeria and association with morphological traits. Morphological traits including the body weight, withers height, body length and chest depth were evaluated using simple standard equipment. The result from sequencing of myostatin gene exons 1 and 3 from extensively managed Red Sokoto goat revealed that exons 1 and 3 regions are polymorphic, with exon 1 being more polymorphic. Three of the obtained five non-synonymous single nucleotide polymorphisms in exon 1 had possible non-neutral effects on protein function. Conversely, one non-synonymous single nucleotide polymorphisms found in exon 3 had possible neutral effect on protein function. Logistic regression revealed one non-synonymous variant at the myostatin gene exon 1 region was associated with body length in extensively managed Red Sokoto goat. This preliminary study on the myostatin gene in Nigerian Red Sokoto goat breed bridged a lack by the provision of valuable reference on the myostatin gene and / or its intervening regions for example exons 1 and 3. Such knowledge would be helpful in further breeding selection strategy and present valuable resources for future studies of goat genomics.

Association entre les polymorphismes exons 1 et 3 du gène de la myostatine et les caractères morphologiques de la race chèvre rouge du Sokoto nigérian

Mots clés supplémentaires
Gène de la myostatine.
Polymorphismes.
Traits morphologiques.
Chèvre rouge Sokoto.
Nigeria.

RÉSUMÉ

Des exons de gènes contrôlent l'information génétique qui est transcrite et traduite en un produit protéique qui produit, directement ou indirectement, des phénotypes observés de caractères. La nécessité de disposer d'informations génétiques, en relation avec les locus qui affectent les caractères de performance, peut être un outil important dans le programme de sélection. La recherche s'est concentrée sur la génétique, l'amplification et le séquençage du gène de la myostatine à partir du sang de la race de chèvre Red Sokoto du Nigéria et sur l'association avec les caractères morphologiques. Les caractères morphologiques, y compris le poids corporel, la hauteur du garrot, la longueur du corps et la profondeur de la poitrine ont été évalués à l'aide d'un équipement standard simple. Le résultat du séquençage des exons 1 et 3 du gène de la myostatine provenant de la chèvre Red Sokoto, largement gérée, a révélé que les régions des exons 1 et 3 sont polymorphes, l'exon 1 étant plus polymorphe. Trois des cinq polymorphismes mononucléotidiques non synonymes obtenus dans l'exon 1 ont eu des effets non neutres possibles sur la fonction protéique. Inversement, un polymorphisme mononucléotidique non synonyme trouvé dans l'exon 3 avait un effet neutre possible sur la fonction de la protéine. La régression logistique a révélé qu'un variant non-synonyme à la région de l'exon 1 du gène de la myostatine était associé à la longueur du corps chez la chèvre rouge Sokoto largement gérée. Cette étude préliminaire sur le gène de la myostatine dans la race chèvre rouge Sokoto au Nigeria a comblé un manque en fournissant une référence valable sur le gène de la myostatine et / ou ses régions intermédiaires, par exemple les exons 1 et 3. Ces connaissances seraient utiles présenter de précieuses ressources pour de futures études sur la génomique caprine.

Information
Cronología del artículo.
Recibido/Received: 22.11.2017
Aceptado/Accepted: 05.05.2018
On-line: 07.04.2019
Correspondencia a los autores/Contact e-mail:
sannitim4real@yahoo.com

INTRODUCTION

Myostatin (*MSTN*) gene, belonging to the Transforming Growth Factor-β superfamily, is a candidate gene implicated in the growth, development and performance of domestic animals (Yu *et al.*, 2007). As a growth factor, it is expressed in muscle that regulates

growth and development by the inhibition of cell cycle progression (McPherron, 1997). Myostatin gene is of importance in domestic animals due to its key role in muscle growth and its potential applications in goat breeding (Alakilli *et al.,* 2012). The *MSTN* gene comprises of three exons and two intervening intron regions. Exons of genes contain the genetic information that is transcribed and translated to the protein product that produces, directly or indirectly, observed phenotypes of traits (Singh *et al.,* 2012). The investigation of the quality of meat and related genes is important (Fontanesi *et al*., 2008). For the roles they play, *MSTN* gene and other genes for example growth hormone (*GH*) gene are considered as candidate genes for meat production traits (Fontanesi *et al*., 2008). Myostatin is considered as one of the most important gene responsible for meat quality traits (Sadkowski *et al.,* 2008). The physiological significance of candidate genes in relation to different phenotypes of growth traits has possible implications in caprine genetic improvement.

Genetic polymorphism is a significant tool for evaluating variations within and across breeds in animal conservation programmes. Polymorphism studies have helped in the detection of several genes and their possible association with functional traits. The morphological traits of animals constitute major concerns in the determination of economic value in animal husbandry. Previous studies showed that, in general, body weight, sex, age, body size, body condition, coat color, breed type and other production factors are important in analysing preferences and economic values of livestock attributes (Kassie *et al*., 2012). Polymorphism in the DNA was reported to be associated with economic traits in livestock species (Gelderman, 1997). Mutation in the *MSTN* gene has been found to be associated with morphological traits in livestock. Growth and other traits of economic values find their relevance in the genetic merit of the animal species. Growth traits are regulated by polygenic genes which may be important candidates for unraveling the genetic variation in economically relevant traits in farm animals (An *et al.,* 2012).

Goats are important livestock species, widely distributed across different geographical regions of the world. Goats have contributed immensely to the resource-poor communities for their livelihood, since they serve as source of food and biological raw materials and play significant socio-economic roles in the lives of rural dwellers (Oseni *et al.,* 2006). The three popular Nigerian goats' breeds are the Red Sokoto, the Sahel and the West African Dwarf goats. The Red Sokoto is the only Nigerian goat breed for which there is a record of systematic attempt to stabilize a particular type (Bourn *et al.,* 1994). Nigeria goats' population was put at 34.5 million goats with a much larger population concentrated in the northern region than in the southern region (Lawal-Adebowale, 2012).

There are fewer studies on small ruminants, particularly goat, as much as are available in cattle (Chukwuka *et al.,* 2010). Previous molecular studies (including diversity studies) have been reported by earlier workers on extensively managed indigenous goat breeds of Nigeria (Adebambo *et al.,* 2011; Okpeku *et al.,* 2011; Sanni *et al.,* 2011; Adefenwa *et al.,* 2013; Sanni *et al.,* 2013; Yakubu *et al.,* 2013; Awotunde *et al.,* 2015). Few studies in the literature have examined the *MSTN* gene in goat species. The *MSTN* gene was investigated because of the important role in muscle growth and its potential applications in goat breeding. Most genetic variations believed to cause phenotypic differences between individuals is represented by single nucleotide polymorphisms. We hypothesised that there is significant association between *MSTN* gene and morphological characteristics in goat. The objective of the study was to provide relevant information from the sequenced exons 1 and 3 regions of *MSTN* gene and identify polymorphisms that were used to evaluate their potential association with identified goat morphology. The study invariably determined the single nucleotide polymorphisms, genetic diversity and predicts possible effect of observed mutations on the structure and functions of protein in myostatin gene at exon 1 and exon 3 regions in Nigerian Red Sokoto goat breed using DNA sequencing analysis.

MATERIAL AND METHODS

Animals and sampling

Blood samples of 5 ml drawn from unrelated Red Sokoto goat breed (RS, n = 55) of Nigeria (**Figure 1**) by jugular venipuncture were collected in sterile tubes containing EDTA as an anticoagulant and stored at -20ºC for DNA extraction. Phenotypic parameters such as body weight (BW), withers height (WH), body length (BL) and chest depth (CD) were evaluated using simple standard equipment, without compromising the animals' welfare. The goats originated from different herds that were reared under the traditional extensive system, where they grazed during the day on natural pasture containing forages such as leucaena (*Leucaena leucocephala*), guinea grass (*Panicum maximum*) and stylo (*Stylosanthes gracilis*) and scavenged on household and kitchen wastes whenever available. The goats mainly from the northern part of the country were transported to the local markets in Abeokuta,

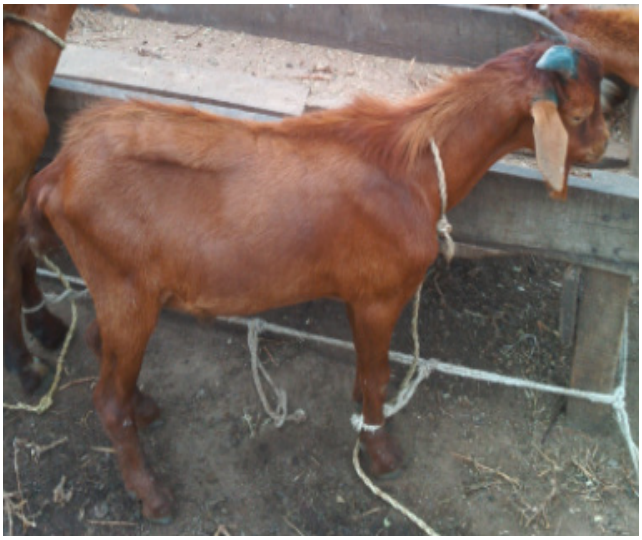

Figure 1. Red Sokoto goat (♂) (Chèvre Sokoto rouge (♂))

Ogun State, belonging to the south western part of the country. The Red Sokoto goats were sourced from local markets in Abeokuta, Nigeria. Informed consent was obtained from each owner before animal inclusion in the study.

DNA extraction, primers, PCR assay and sequencing

Caprine genomic DNA was successfully isolated from blood samples at the Animal Breeding and Genetics Laboratory, Federal University of Agriculture, Abeokuta, Nigeria using *Quick-gDNA*™ MiniPrep Genomic DNA Purification Kit (Zymo Research, USA) according to the manufacturer's protocol. The quality and quantity of DNA were checked visually on 2% agarose gel electrophoresis and by spectrophotometric method. The exons 1 and 3 regions of caprine *MSTN* gene were amplified by designing primers in exon-intron boundaries. The primer sequences (forward, reverse) targeting exons 1 and 3 regions of *MSTN* gene used for the study and their annealing temperatures are presented in **Table I**. The PCR primers were designed using web interface of Primer3 (http://frodo.wi.mit.edu/). The PCR assays were performed in a programmable thermal cycler. The PCR amplification reaction for exon 1 region of *MSTN* gene consist of 35 cycles of 95 °C for 30 sec, 56 °C for 1 min and 72 °C for 2 min. The amplification reaction for the exon 3 region consist of 35 cycles of 95 °C for 30 sec, 54°C for 1 min and 72 °C for 2 min. The gels were analyzed by 2% agarose gel electrophoresis. All other protocols and reagents are standard from Qiagen. Selected PCR products were bidirectionally sequenced using Sanger sequencing with respective forward and reverse primer to detect if there were any variations at the nucleotide level.

Multiple sequence alignment and genetic diversity

Obtained sequences (40 and 43 sequences each for *MSTN* gene exons 1 and 3 respectively) were aligned using the *Phrap* programme for contig assembling (Rieder *et al.*, 1998) as implemented in CodonCode aligner DNA sequence assembly version 5.1.5 (http://www.codoncode.com/aligner.htm). Heterozygous sites were screened and determined by BioEdit software version 7.2.3 (Hall, 1999). Polymorphisms were identified by the visual inspection of the aligned sequences. The coding DNA sequences of exons 1 and 3 regions of *MSTN* gene were conceptually translated to amino acid sequences in all six translation frames using the same software. The *MSTN* gene exons 1 and 3 consensus sequences of the RS goat were each aligned with a publicly available 6356 base pairs of *Capra hircus MSTN* gene from the GenBank at the NCBI with Accession number EF591039.1. The *MSTN* gene exon 1 region span from 1201-1698 base pairs while the *MSTN* gene exon 3 region span from 5818-6260 base pairs of the *Capra hircus MSTN* gene (EF591039.1). DnaSP version 5.10.01 (Librado and Rozas, 2009) was used to sort haplotypes and analyse haplotype diversity (Hd, Nei, 1987), average number of nucleotide differences (K, Tajima, 1983) and nucleotide diversity (π, Hd, Nei, 1987). MEGA version 6.06 (Tamura *et al.*, 2013) was used to define nucleotide and amino acid haplotypes by cross-validation.

Predicting effect of mutations on protein function

Screening for Non-Acceptable Polymorphisms (SNAP) analysis (Bromberg *et al.*, 2008) was conducted to predict effect of mutations on protein function using the web-based tool called SNAP software developed by Yana Bromberg at the Rost Laboratory, Columbia University, New York (https://rostlab.org/services/snap/). Screening for Non-Acceptable Polymorphisms is a method for evaluating effects of single amino acid substitutions on protein function. As a neural-network-based method, SNAP uses *in silico* derived protein information (e.g. secondary structure, conservation, solvent accessibility, etc.) to make predictions regarding functionality of mutated proteins. The SNAP analysis was performed with aligned amino acid sequences from exons 1 and 3 regions of *MSTN* gene in the extensively managed Red Sokoto goat breed. The default reliability index of 0 was used from a scale with index between 0 and 9 while expected accuracy was set at a minimum threshold of 50%.

Logistic regression analysis

The effects of non-synonymous mutation on the explanatory variables, being the phenotypic parameters (BW, WH, BL and CD) of the RS goat breed were assessed by a logistic regression analysis using the R software (version 3.0.2) (R software, 2013). The response variables were the single nucleotide polymorphisms found in exons 1 and 3 regions of *MSTN* gene. Statistical differences based on the data were assessed using a confidence level of $P<0.05$ or 95% confidence interval. The R software was also used to analyse the differences in genotypic and allelic frequencies at the polymorphic loci using χ^2-test of the Generalised Linear Models function in logit regression (where the outcome variable undergoes some transformation to enable the model to take the form of a linear combina-

Table I. Primers, amplified regions, annealing temperature and use of the polymerase chain reaction product (Primers, régions amplifiées, température de recuit et utilisation du produit de réaction en chaîne par polymérase)

Primer pair	F: Forward sequence (5´-3´) R: Reverse sequence (3´-5´)	Primer annealing region	Amplified region	Annealing temperature (°C)	Use of the PCR product
1	1F: AGGCATTAACGTTTGGCTTG	Exon 1	< 1 Kb	56	Sequencing
	1R: ACACTAGAACAGCAGTCAGCAGA	Exon 1			Sequencing
2	2F: TCTTTAATAATGACTCCCTGCG	Exon 3	< 1 Kb	54	Sequencing
	2R: GAACACCCACAGCGATCTACT	Exon 3			Sequencing

F = forward; R = reverse

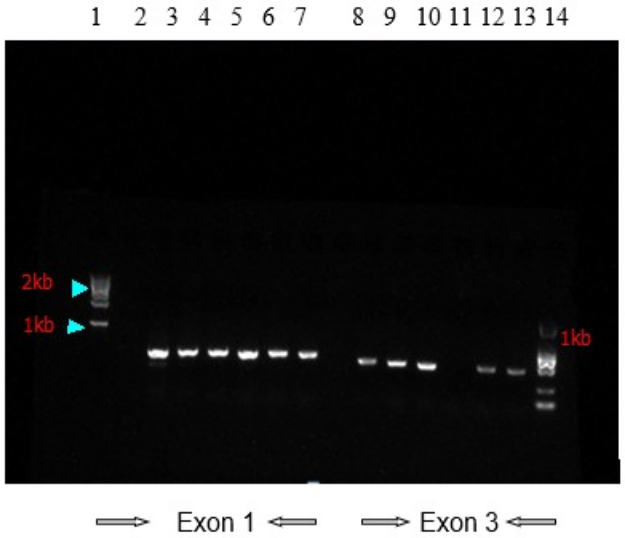

Figure 2. Agarose gel elecrophoresis of specific polymerase chain reaction for Myostatin gene exon 1 (left) and exon 3 (right). Marker standard = 2 Kb standard to the left of the gel picture and 1Kb to the right (from the gel, the Myostatin gene exon 1 is the first 6 bands and exon 3 is the last five bands (one band failed), the overall size is below 1Kb) (Électrophorèse sur gel d'agarose d'une réaction en chaîne par polymérase spécifique pour l'exon 1 du gène de la myostatine (à gauche) et l'exon 3 (à droite). Marqueur standard = 2 Kb standard à gauche de l'image gel et 1Kb à droite (à partir du gel, le gène Myostatin exon 1 est le premier 6 groupes et l'exon 3 est les cinq dernières bandes (un groupe a échoué), la taille globale Est inférieur à 1 Ko)

tion) and to analyse the relationship between single nucleotide polymorphisms genotypes and phenotypic traits in the goats.

Application of different tools such as single nucleotide polymorphism genotyping, BioEdit software, Molecular Evolutionary Genetics Analysis (MEGA) software, DnaSP software, Screening for Non-Acceptable Polymorphisms software and the R software were employed to clarify, verify and validate the findings of the study.

RESULTS

Genetic differentiation and diversity

The genetic differentiation and diversity parameters in exon 1 and 3 regions of *MSTN* gene in extensively managed Red Sokoto goat are presented in **Table II**. The 497 base pairs sequence size of *MSTN* gene exon 1 region arising from the 40 sequences used in the RS goat generated 435 invariable (or monomorphic) sites. A total of 5 polymorphic (or variable) sites, resulting in 3 singleton variable sites with two variants and 2 parsimony informative sites with two variants were found at the *MSTN* exon 1 region in the RS goat. The numbers of haplotypes found at the *MSTN* exon 1 region in RS goats were 6, with sequence conservation of 0.969. The RS *MSTN* gene exon 3 sequence size consists of 442 base pairs. One polymorphic (or variable) site, resulting in a singleton variable site and 0 parsimony informative sites was found at the exon 3 region of *MSTN* gene in the RS goat. Two haplotypes with sequence conservation of 0.971 were found at the *MSTN* exon 3 region in the RS goat.

Single nucleotide polymorphism and agarose gel electrophoresis

A total of five single nucleotide polymorphisms were identified from the exon 1 region of *MSTN* gene (**Table III**), one transition mutation (*A80G*) and four transversion mutations (*A32T, C84G, T85G* and *T473G*). A total of 43 sequences in RS goat were used for the single nucleotide polymorphism analysis from the exon 3 region of *MSTN* gene (**Table III**). One transversion mutation (*C49A*) was found. The agarose gel electrophoresis of specific polymerase chain reaction for exons 1 and 3 of *MSTN* gene with molecular markers in RS goat is shown in **Figure 2**. The electropherogram overall band reads for both exons 1 and 3 were below 1 Kb.

Prediction of the possible effect of observed mutations on protein function

The SNAP analysis conducted to predict the effects of mutations on protein function found five non-synonymous polymorphisms at the exon 1 and one non-synonymous polymorphism at the exon 3 regions of *MSTN* gene (**Table IV**). The functional significance of the non-synonymous polymorphisms was accessed *in silico* using SNAP programme. Three of the obtained five non-synonymous polymorphisms at the exon 1 were predicted to have a possible non-neutral effect on protein function. Two non-synonymous polymorphisms at the exon 1 region had a possible neutral effect on protein function. One non-synonymous polymorphism in exon 3 was predicted to have a possible neutral effect on protein function with reliability index of 2 and expected accuracy of 69%.

Effects of single nucleotide polymorphisms in myostatin gene on morphological traits

The effects of single nucleotide polymorphisms found in the exons 1 and 3 regions of *MSTN* gene on morphological traits in the extensively managed RS goat breed were assessed using logistic regression (**Ta-**

Table II. Genetic differentiation and diversity in Myostatin gene in extensively managed Red Sokoto goat (Différenciation génétique et diversité du gène Myostatine dans une chèvre Sokoto à gestion intensive)

MSTN gene	N	I	P	SP	PI	H	Hd	π	K	C
Exon 1	40	435	5	3	2	6	0.428	0.00126	0.554	0.969
Exon 3	43	377	1	1	0	2	0.047	0.00012	0.047	0.971

MSTN, Myostatin; N, Number of sequence; I, Invariable site; P, Polymorphic site; SP, Singleton variable sites; PI, Parsimony informative site; H, Number of Haplotype; Hd, Haplotype diversity; π, Nucleotide diversity; K, Average no. nucleotide differences; C, Sequence conservation.

Table III. Single nucleotide polymorphisms in Myostatin gene in extensively managed Red Sokoto goat (Polymorphismes à un seul nucléotide dans le gène Myostatine dans une chèvre Sokoto à gestion intensive).

MSTN gene	Number of sequences	Number of SNPs	Mutated nucleotides	Amino acid change	Chemical property	Allele	DNA codon change
Exon 1	40	5	*A32T*	Lys 11Met (*K11M*)	+ charge>polar, non-charge	*37A, 3T*	AAG>ATG
			A80G	Gln27Arg (*Q27R*)	Polar, non-charge>+ charge	*34A, 6G*	CAA>CGA
			C84G	Ile28Met (*I28M*)	Non-polar, aliphatic>polar, non-charge	*39C, 1G*	ATC>ATG
			T85G	Phe29Val (*F29V*)	Aromatic>non-polar, aliphatic	*39T, 1G*	TTT>GTT
			T473G	Leu158Arg (L158R)	Non-polar, aliphatic>+ charge	*39T, IG*	CTG>CGG
Exon 3	43	1	*C49A*	His17Asn (*H17N*)	+ charge>polar, non charge	*42C, 1A*	CAT>AAT

MSTN = Myostatin; + = positive; SNP = single nucleotide polymorphisms

ble V). It was found that one non-synonymous variant from the exon 1 region of *MSTN* gene was associated with body length in RS goat under a model fit (OR = 0.62 [0.30, 0.86] 95% (odds ratio and 95% confidence interval)) with significant P = 0.0003. The association of the other single nucleotide polymorphism variants from the exons 1 and 3 regions of *MSTN* gene were not significant (P>0.05). Logistic regression revealed the result of predicting the binary response of single nucleotide polymorphisms (five single nucleotide polymorphisms at the exon 1 and one single nucleotide polymorphism at the exon 3 regions of *MSTN* gene) found on the explanatory variables i.e. morphological traits (BW, WH, BL and CD) of the Nigerian RS goat breed. Logistic regression retained BL as the only variable included in the equation and excluded BW, WH and CD as variables not in the equation.

Effects of sex and single nucleotide polymorphisms on morphological traits

The effects of sex and single nucleotide polymorphisms on the morphological traits in RS goat at the exons 1 and 3 regions are presented in **Table VI**. The sex of the goat had no significant effect (P>0.05) on the measured morphological traits at the *MSTN* gene exons 1 and 3 regions. The effect of the identified single nucleotide polymorphisms from the exons 1 and 3 regions of *MSTN* gene were not significant (P>0.05) on the RS goat's morphological traits measured. However, significant effects (P<0.05) were found based on K11M single nucleotide polymorphism on the BL, CD and WH at the exon 1 region and for H17N single nucleotide polymorphism on the BW, WH and CD at the exon 3 region of *MSTN* gene.

DISCUSSION

The polymorphisms of the *MSTN* gene have been implicated to have important economic consequences in animal husbandry (Li *et al.,* 2006). Improving the meat productivity in the RS goat breed is important while retaining its other favourable characteristics. In this study, investigation was based on muscle production in relation to single nucleotide polymorphisms (targeting exons 1 and 3) in the *MSTN* gene. It was observed that there was high degree of conservation in goat *MSTN* gene.

The investigated sequences from exons 1 and 3 regions of *MSTN* gene in RS goat breed revealed evidences of polymorphisms. The moderate degrees of nucleotide variation with five single nucleotide polymorphisms identified within the exon 1 of *MSTN* gene in RS goat with five non-synonymous changes suggest

Table IV. Prediction of functional consequences and in silico scores of non-synonymous mutations in exons 1 and 3 regions of Myostatin gene in extensively managed Red Sokoto goat (Prédiction des conséquences fonctionnelles et scores in silico de mutations non synonymes dans les régions exons 1 et 3 du gène Myostatine dans la chèvre Sokoto à gestion intensive)

MSTN gene	nsSNP	Prediction	Reliability index	Expected accuracy (%)
Exon 1	*K11M*	Neutral	0	53
	Q27R	Non-neutral	0	58
	I28M	Neutral	5	89
	F29V	Non-neutral	1	63
	L158R	Non-neutral	2	70
Exon 3	*H17N*	Neutral	2	69

MSTN = Myostatin, nsSNP = Non-synonymous single nucleotide polymorphism, K = Lys, M = Met, Q = Gln, R = Arg, I = Ile, F = Phe, V = Val, L = Leu, H = His, N = Asn.

Table V. Identified variants from the exons 1 and 3 regions of Myostatin gene and association between non-synonymous variants and body length in extensively managed Red Sokoto goat using a logistic regression (Des variants identifiés des régions Exons 1 et 3 du gène de la Myostatine et l'association entre les variantes non synonymes et la longueur du corps dans la chèvre Red Sokoto à gestion intensive en utilisant une régression logistique)

MSTN gene	Variants	Nucleotide change	MAF	Coefficients (b) [intercept]	OR [95% CI]	P-value
Exon 1	*K11M*	A>T	0.075	-0.48 [41.15]	0.62 [0.30; 0.86]	0.0003
	Q27R	A>G	0.15	-0.04 [2.69]	0.96 [0.86; 1.04]	0.3164
	I28M	C>G	0.025	-0.03 [-0.97]	0.97 [0.76; 1.17]	0.7852
	F29V	T>G	0.025	-0.03 [-0.97]	0.97 [0.76; 1.17]	0.7852
	L158R	T>G	0.025	0.17 [-11.36]	1.08 [0.89; 1.36]	0.4289
Exon 3	*H17N*	C>A	0.023	0.18 [-23.85]	1.19 [0.97; 1.88]	0.0986

MSTN, Myostatin; MAF, minor allele frequency; OR, odds ratio; CI, confidence interval

that non-synonymous substitutions exceed synonymous substitutions indicating that the evolution of this protein might have arisen through positive selection in this domestic animal.

The coding sequence at the exon 1 region of *MSTN* gene in RS goat was found to be moderately polymorphic and may be regarded as a 'moderate mutational hot spot' when compared to the exon 3 region. This shows that the exon 1 is more polymorphic than the exon 3 of *MSTN* gene in RS goat breed. Khichar *et al.* (2016) reported they found two polymorphic sites in the molecular characterisation of exon 3 of *MSTN* gene which was a low genetic variation in the target region in Marwari goat. Soufy *et al.* (2009) reported that they found polymorphism in *MSTN* gene exon 3 in Sanjabi sheep and native Kermanian cattle. Similar nucleotide variations in different species were valuable insight in elucidating the observed variations within these particular regions in the *MSTN* gene. Further research would be needed to demonstrate the putative functional role of the identified goat *MSTN* exon single nucleotide polymorphisms and their expression in skeletal muscle.

Myostatin gene polymorphism and its possible influence on various phenotypic traits have been reported in many species, and some possible associations between production traits and polymorphism have been established. This *MSTN* gene, located on chromosome 2 and the single nucleotide polymorphism DQ530260: g6223G>A in intron 2, has been shown to affect muscularity in sheep (Kijas *et al.*, 2007). Single nucleotide polymorphisms were reportedly identified in the non-coding regulatory regions of ovine and porcine *MSTN* gene (Dybus *et al.*, 2013). Some of them were reported to influence the expression levels of *MSTN* gene, which can be correlated with muscle

Table VI. Effects of sex and single nucleotide polymorphism in exons 1 and 3 regions of Myostatin gene on the means with standard errors of phenotypic traits in extensively managed Red Sokoto goat (Effets du sexe et du polymorphisme nucléotidique unique dans les régions exons 1 et 3 du gène Myostatine sur les moyens avec des erreurs types de traits phénotypiques dans la chèvre Sokoto à gestion intensive)

MSTN gene	Factor	Level	N	Body weight (kg)	Body length (cm)	Chest depth (cm)	Withers height (cm)
Exon 1	Sex	Buck	38	17.61±1.30	102.04±1.80	28.84±0.61	61.67±1.06
		Doe	2	27.00±6.00	99.06±2.54	29.00±4.0	57.50±4.50
	SNPs						
	K11M	*KK*	37	18.51±1.37	103.22±1.67[a]	29.23±0.59[a]	62.22±0.99[a]
		MM	3	12.67±2.33	85.51±0.85[b]	24.13±1.49[b]	52.17±3.17[b]
	Q27R	*QQ*	34	18.71± 1.43	102.59±1.86	29.19±0.65	61.91±1.18
		RR	6	14.5±2.73	97.96±4.52	26.90±1.32	58.92±1.32
	I28M	*II*	39	18.13±1.33	101.96±1.76	28.99±0.59	61.55±1.06
		MM	1	16.00±0.00	99.06±0.00	23.00±0.00	58.00±0.00
	F29V	*FF*	39	18.13±1.33	101.96±1.76	28.99±0.59	61.55±1.06
		VV	1	16.00±0.00	99.06±0.00	23.00±0.00	58.00±0.00
	L158R	*LL*	39	18.23±1.32	101.67±1.74	28.84±0.61	61.42±1.05
		RR	1	12.00±0.00	110.49±0.00	29.00±0.00	63.00±0.00
Exon 3	Sex	Buck	2	27.00±6.00	99.06±2.54	29.00±4.00	57.50±4.50
		Doe	41	18.56±1.23	101.43±1.89	28.73±0.55	61.57±1.06
	H17N	*HH*	42	18.59±1.19[b]	100.89±1.80	28.57±0.53[b]	61.04±0.99[b]
		NN	1	34.00±0.00[a]	119.38±0.00	36.00±00[a]	76.00±0.00[a]

[a,b] Means with different superscripts in the same column within factor differed significantly ($p<0.05$), N = Number of observations, SNP = single nucleotide polymorphisms

mass, growth and carcass performance traits (Dall'Olio *et al.,* 2010). It was also known to be associated with muscularity, growth, and meat quality traits in the promoter region of pig (Stinckens *et al.,* 2008). This suggests that polymorphisms in the exons could contribute to muscularity in this goat breed.

The SNAP analysis conducted was able to predict the effect of mutations on protein function. Genetic variations are majorly represented by single nucleotide polymorphisms. Those single nucleotide polymorphisms found in the coding regions of *MSTN* gene are often synonymous, causing no change in amino acid of the encoded protein sequence. These predicted neutral effects of mutation on protein function in the coding regions of exons 1 and 3 of *MSTN* gene in RS goat breed indicate that the resulting point-mutated proteins are not functionally discernible from the wild-type. This means that the mutation in the resulting breed and the wild- type do not differ in function.

Conversely, the non-neutral prediction of protein function at the exon 1 region of *MSTN* gene in the RS goat indicates that the resulting point-mutated proteins and wild-type differ in function. Factors such as breeding and/or source of location of the RS goat breed used for the study may implicate such non-neutral possible outcome in identified mutated goats since the breeding history of the goats sourced from different locations was not known. It is possible that some of the RS goat breed sampled from the markets may have been products of cross breeding and were mainly from the large expanse of Northern Nigeria.

Logistic regression analysis identified one non-synonymous variant in exon 1 to be associated with body length in the RS goat breed. Genetic variations associated with morphological distinctions between individuals is represented by single nucleotide polymorphisms. Zhang et al. (2013) reported that they found significant association between the genetic variation within 5'-UTR and exon I in the *MSTN* gene and growth traits (body weight, body height, body length, and chest circumference) in Anhui white goat and Boer goat used in their study. Caprine *MSTN* gene is one of the few candidate genes that play important role in bone formation, birth weight, body condition, and muscle growth Khichar *et al.* (2016). As a candidate gene, *MSTN* gene could be utilized as genetic marker and is responsible for relatively large proportion of genetic variation (Lande, 1981). Owing to the biological function, the *MSTN* gene represents a notable candidate gene that may influence growth traits in goats. The study has determined whether or not the five mutations in the exon 1 and the one mutation in exon 3 regions of *MSTN* gene were influencing the morphology of the RS goat breed and the possible effect of such mutations on selected qualities of linear body measurements and growth traits. Only one of the mutations at the exon 1 region was found to be associated with body length in the RS goat breed. However, we suggest further investigation of these results before they are used in goat breeding and genetics.

The data from the exons 1 and 3 regions of *MSTN* gene provide evidence of polymorphism in RS goat breed. Various studies have confirmed several polymorphisms being found for *MSTN* gene. Myostatin gene has been reported to be highly variable within beef cattle (Dunner *et al.,* 2003). In a study conducted by Guifen *et al.* (2014) on the identification of single nucleotide polymorphism and analysis in part of exon 3 of beef cattle *MSTN* gene and the variation among breeds, the researchers found three mutative sites at the exon 3 region of *MSTN* gene which provided strong evidence that the sites might be some invalid mutation in the cattle breeds used for their study. The result from the present study provided evidence that four mutative sites (out of the five found) at the exon 1 region of *MSTN* gene might be some invalid mutation to the researched breed (RS goat). .

In this study, five mutative sites has been found at the exon 1 region of *MSTN* gene and one mutative site at the exon 3 region of *MSTN* gene in RS goat breed. However, there was no difference between sexes and no effect of single nucleotide polymorphisms on body weight in the RS goat breed. The mutation occurring in the coding region of *MSTN* gene was described to result in body weight gain, but was associated with increased mortality rates (Dybus *et al.,* 2013). Consequently, *MSTN* gene is probably pleiotropic in nature (Dybus *et al.,* 2013). Polymorphism studies have helped in the detection of several genes and their possible association with body parameters. Economically-important growth traits in goats and their variations are predominantly attributed to possible association with different factors especially genetic factors. The increasing financial value of goat breeding worldwide is a strong reason for researchers to give necessary attention to this species. Genetic markers could improve incomes from animal breeding in the future (Ye *et al.,* 2007). The results from this study suggest that the mutation located at the exon 1 region of *MSTN* gene could be considered a potential genetic marker for body length in RS goat breed. These data provide useful information that the RS goat breed have a polymorphism in exon 1 for myostatin locus. The present study is preliminary, in which the morphological structure of the gene encoding myostatin in the RS goat breed was analysed.

CONCLUSIONS

The focus of the study was on the association between *MSTN* gene exon 1 and 3 polymorphisms and morphological traits in Red Sokoto goat breed of Nigeria. Through the application of different tools, the study demonstrated that nucleotide variations in goat species were valuable insight in elucidating the observed variations within particular regions in the myostatin gene. Exons 1 and 3 regions of *MSTN* gene in the Red Sokoto goat breed are polymorphic, with exon 1 being more polymorphic. Neutral substitutions and non-neutral substitution could occur in exons 1 region of *MSTN* gene in the Red Sokoto goat breed. Mutation in exon 1 of *MSTN* gene was associated with body length in Red Sokoto goat breed. This preliminary study on the Nigerian Red Sokoto goat breed bridged a gap; providing baseline information and valuable reference on the *MSTN* gene and / or its intervening regions for example exons 1 and 3. Such knowledge

would be helpful in further breeding selection strategy and present valuable resources for future studies of goat genomics.

ACKNOWLEDGMENTS

The contribution of Prof Wen Wang and collaborating partners at the State Key Laboratory for Genetic Resources and Evolution, Chinese Academy of Sciences, Kunming Institute of Zoology, China is much appreciated.

BIBLIOGRAPHY

Adebambo, AO, Adebambo, O, Williams, JL, Blott, S & Urquart B 2011, 'Genetic distance between two popular Nigerian goat breeds used for milk production', *Livestock Research for Rural Development, vol. 23, no. 26, viewed 11* November 2015, <http://www.lrrd.org/lrrd23/2/adeb23026.htm>.

Adefenwa, MA, Agaviezor, BO, Peters, SO, Wheto, M, Ekundayo, OJ, Okpeku, M, Oboh, BO, Adekoya, KO, Ikeobi, CON, De Donato, M, Thomas, BN & Imumorin, IG 2013, 'Novel intron 2 polymorphism in the melanophilin gene is in Hardy-Weinberg equilibrium and is not associated with coat color in goats', *Open Journal of Genetics,* vol. 3, pp. 195-200.

Alakilli, SYM, Mahrous, KF, Salem, LM & Ahmed, ES 2012, 'Genetic polymorphism of five genes associated with growth traits in goat', *African Journal of Biotechnology,* vol. 11, no. 82, pp. 14738-48.

An, X, Zhao, H, Bai, L, Hou, J, Peng, J, Wang, J, Song, Y & Cao, B 2012, 'Polymorphism identification in the goat *THRSP* gene and association analysis with growth traits', *Arch Tierzucht,* vol. 55, no. 1, pp, 78-83.

Awotunde, EO, Bemji, MN, Olowofeso, O, James, IJ, Ajayi, OO & Adebambo, AO 2015, 'Mitochondrial DNA sequence analyses and phylogenetic relationships among two Nigerian goat breeds and the South African Kalahari Red', *Animal Biotechnology,* vol. 26, pp. 180-87.

Bourn, D, Wint, W, Blench, R & Woolley, E 1994, 'Nigerian livestock resources survey', *World Animal Review,* vol. 78, pp. 49-58.

Bromberg, Y, Yachdav G & Rost, B 2008, 'SNAP predicts effect of mutations on protein function', *Bioinformatics,* vol. 24, pp. 2397-98.

Chukwuka, OK, Okoli, IC, Okeudo, NJ, Opara, MN, Herbert, U, Ogbuewu, IP & Ekenyem, BU 2010, 'Reproductive potentials of West African Dwarf sheep and goat: A Review', *Research Journal of Veterinary Sciences,* vol. 3, no. 2, pp. 86-100.

Dall'Olio, S, Fontanesi, L, Costa, LN, Tassinari, M, Minieri, L & Falaschini, A 2010, 'Analysis of horse myostatin gene and identification of single nucleotide polymorphisms in breeds of different morphological types', *Journal of Biomedicine and Biotechnology,* vol. 542945, pp. 1-11, viewed 8 April 205,<hhtp//www.hindawi.com>.

Dunner, S, Miranda, ME, Amigues, Y, Cañón, J, Georges, M, Hanset, R, Williams, J & Ménissier, F 2003, 'Haplotype diversity of the myostatin gene among beef cattle breeds', *Genetics, Selection, Evolution,* vol. 35, pp. 103–18.

Dybus, A, Proskura, WS, Sadkowski, S & Pawlina, E 2013, 'A single nucleotide polymorphism in exon 3 of the Myostatin gene in different breeds of domestic pigeon (*Columba livia* var. *domestica*)', *Veterinarni Medicina,* vol. 58, no. 1, pp. 32–38.

Fontanesi, L, Tazzoli, M, Scotti E & Russo, V 2008, 'Analysis of candidate genes for meat production traits in domestic rabbit breeds', *9th World Rabbit Congress – June 10-13, 2008 – Verona – Italy,* pp. 79-84.

Gelderman, H 1997, 'Investigations on inheritance of quantitative characters in animals by gene markers', *Methods Theoret. Appl. Genet.,* vol. 46, pp. 319-30.

Guifen, L, Fachun, W, Enliang, S, Xiaomu, L, Xiuwen, T & Haijian, C 2014, 'SNP identification and analysis in part of exon 3 of beef cattle *MSTN* gene and variation among breeds', *Journal of Animal and Veterinary Advances,* vol. 13, no. 3, pp. 174-77.

Hall, TA 1999, 'Bioedit: A User-Friendly Biological Sequence Alignment Editor and Analysis Programme for Windows 95/98/NT'. *Nucleic Acids Symposium Series,* vol. 41, pp. 95-98.

Kassie, GT, Abdulai, A & Wollny, C 2012, 'Estimating the worth of traits of indigenous breeds of cattle in Ethiopia', In: Analysis of genetic variation in animals, InTech Publisher, Janeza Trdine 9, 51000 Rijeka, Croatia, pp. 337-360.

Khichar, JP, Gahlot, GC, Agrawal, VK, Kiran, Dewna AS, Prakash & Ashraf M 2016, 'Molecular characterization of exon 3 of caprine myostatin gene in Marwari goat', *Veterinary World,* vol. 9, no. 6, pp. 676-79.

Kijas, JW, McCulloch, R, Edwards, JEH, Oddy, VH, Lee, SH & van der Werf, J 2007, 'Evidence for multiple alleles effecting muscling and fatness at the *Ovine GDF8* locus', *BMC Genetics,* vol. 8, pp. 1–11.

Lande, R 1981, 'The minimum number of gene contributing to qualitative variation between and within populations', *Gene,* vol. 99, pp. 541-53.

Lawal-Adebowale, OA 2012, 'Dynamics of ruminant livestock management in the context of the Nigerian agricultural system', *Livestock Production*, pp. 61-80.

Li, XL, Wu, ZL, Liu, ZZ, Gong, YF, Zhou, RY & Zheng, GR 2006, 'SNP identification and analysis in part of intron 2 of goat *MSTN* gene and variation within and among species', *Journal of Heredity,* vol. 97, pp. 285-89.

Librado, P & Rosaz, J 2009, DnaSP v5: 'A software for comprehensive analysis of DNA polymorphism data', *Bioinformatics,* vol. 25, pp. 1451-52.

Mcpherron, AC, Lawler, AM & Lee, SJ 1997, 'Regulation of skeletal muscle mass in mice by a new TGF-Beta superfamily member', *Nature* vol. 387, pp. 83–90.

Nei, M 1987, *Molecular Evolutionary Genetics*, New York: Columbia University Press.

Okpeku, M, Peters, SO, Ozoje, MO, Adebambo, OA, Agaviezor, BO, O'Neill, MJ & Imumorin, IG 2011, 'Preliminary analysis of microsatellite-based genetic diversity of goats in Southern Nigeria', *Animal Genetic Resources,* vol. 49, pp. 33–41.

Oseni, S, Sonaiya, B, Omitogun, G, Ajayi, A & Muritala, I 2006, 'West African Dwarf goat production under village conditions: characterisation and the establishment of breed standards', *Conference on International Agricultural Research for Development*, University Of Bonn, Tropentag 2006 October 11-13, 2006.

R software 2013, 'The R Foundation for Statistical Computing.'

Rieder, MJ, Taylor, SL, Tobe, VO & Nickerson, DA 1998, 'Automating the identification of DNA variations using quality-based fluorescence re-sequencing: analysis of the human mitochondrial genome', *Nucleic Acids Research,* vol. 26, no. 4, pp. 967–73.

Sadkowski, T, Jank, M, Zwierzchowski, L, Siadkowska, E, Oprzdek, J & Moty, T 2008, 'Gene expression profiling in skeletal muscle of holstein-friesian bulls with single-nucleotide polymorphism in the Myostatin gene 5-flanking region', *Journal of Applied Genetics,* vol. 49, pp. 237-50.

Sanni, MT, Adefenwa, MA, Agaviezor, BO, Ikeobi, CON, Wheto, M, Okpeku, M, Yakubu, A, Takeet, MI, De Donato, M & Imumorin, IG 2011, 'Molecular survey of *Trypanosoma vivax* infection in Nigerian goats', *Journal of Animal Science,* vol. 89, no. 1, p. 654.

Sanni, MT, Onasanya, GO, Adefenwa, MA, Yakubu, A, Ikeobi, CON, Adebambo, OA, Talabi, AO, Ozoje, MO, Wheto, M, Takeet, MI, Peters, SO, De Donato, M, Thomas, BN & Imumorin, IG 2013, 'Molecular diagnosis of subclinical African *Trypanosoma vivax* infection and association with physiological indices and serum metabolites in extensively managed goats', *Open Journal of Veterinary Medicine,* vol. 3, pp. 39-45.

Singh, LV, Tripathi, V, Sharma, R, Pandey, AK, Maitra, A & Mishra, BP 2012, 'Genetic polymorphism of *CAPN1* gene in Sirohi goat', *International Journal of Meat Science,* vol. 2, pp. 13-19.

Soufy, B, Mohammadabadi, MR & Shojaeyan, K 2009, 'Evaluation of Myostatin gene polymorphism in Sanjabi sheep by PCR-RFLP method', *Animal Science Researches,* vol. 19, no. 1, pp. 81–89.

Stinckens, A, Luyten, T & Bijttebier, J 2008, 'Characterization of the complete porcine *MSTN* gene and expression levels in pig breeds differing in muscularity', *Animal Genetics,* vol. 39, no. 6, pp. 586–96.

Tajima, F 1983, 'Evolutionary relationship of DNA sequences in finite populations', *Genetics,* vol. 105, no. 2, pp. 437–60.

Tamura, K, Stecher, G, Peterson, D, Filipski, A & Kumar, S 2013. 'MEGA6: Molecular Evolutionary Genetics Analysis Version 6.0', *Molecular Biology and Evolution,* vol. 30, pp. 2725-29.

Yakubu, A, Salako, AE, De Donato, M, Takeet, MI, Peters, SO, Adefenwa, MA, Okpeku, M, Wheto, M, Agaviezor, BO, Sanni, MT, Ajayi, OO, Onasanya, GO, Ekundayo, JO, Ilori, BM, Amusan, SA & Imumorin, IG 2013, 'Genetic diversity in exon 2 of the Major Histocompatibility Complex Class II *DQB1* locus in Nigerian goats', *Biochemical Genetics,* vol. 51, no. 11-12, pp. 954-66.

Ye, X, Brown, SR, Nones, K, Coutinho, LL, Dekkers, JCM & Lamont, SJ 2007, 'Associations of Myostatin gene polymorphisms with performance and mortality traits in broiler chickens', *Genetics Selection Evolution,* vol. 39, pp. 73–89.

Yu, L, Tang, H, Wang, J, Wu, Y, Zou L, Jiang Y, Wu, C & Li, N 2007, 'Polymorphisms in the 5' regulatory region of myostatin gene are associated with early growth traits in Yorkshire pigs', *Science in China Series C: Life Sciences,* vol 50, no. 5, pp. 642–47.

Zhang, G, Ding, F, Wang, J, Dai, G, Xie, K, Zhang, L, Wang, W & Zhou, S 2011, 'Polymorphism in exons of the Myostatin gene and its relationship with BW traits in the Bian chicken', *Biochemical Genetics,* vol. 49, pp. 9–19.

Zhang, ZJ, Ling, YH, Wang, LJ, Hang, YF, Guo, XF, Zhang, YH, Ding, JP & Zhang, XR 2013, 'Polymorphisms of the myostatin gene (*MSTN*) and its relationship with growth traits in goat breeds', *Genetics and Molecular Research,* vol. 12, no. 2, pp. 965-71.