

for 2 min. That of exon 3 consists 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. Selected PCR products were bidirectionally sequenced at the Sain Biotechnology (Shanghai) Co., Ltd, China using Sanger sequencing.

BLAST (Basic Local Alignment Search Tool) was conducted in NCBI (National Centre for Biotechnology Information) using BlastX options to retrieve homologous sequences relative to other species from the GenBank (<http://blast.ncbi.nlm.nih.gov/Blast/>). Amino acid sequences that were not available at the NCBI were retrieved from the Universal Protein Resources (UniProt) database. Sequences were aligned with those of the reported exon 1 and exon 3 *MSTN* genes of selected species obtained from

GenBank using ClustalW algorithm in BioEdit Sequence Alignment Editor Software (version 7.2.3). The phylogenetic analysis was conducted using MEGA version 6.06.

Results and Discussion

The results of the percent amino acid sequence identity of Nigerian Red Sokoto goat at the exons 1 and 3 regions of *MSTN* gene with selected animal species are presented in Table 1. The sequence identity of the exon 1 region of *MSTN* gene of Nigerian RS goat with published species ranged from 88 - 100% in ruminants and 78 - 92% in monogastric and nonruminants. The percent amino acid sequence identity of the Nigerian RS goat with selected animal species at the exon 3 region of *MSTN* gene ranged from 95 - 100% in ruminants and with little variation from 97 - 98% in other nonruminants.

Table 1: Percent amino acid sequence identity at exons 1 and 3 regions of *MSTN* gene in Nigerian goat (Red Sokoto) with selected animal species

Common name	Species	<i>MSTN</i> exon 1		<i>MSTN</i> exon 3	
		Accession	Identity (%)	Accession	Identity (%)
Goat	<i>Capra hircus</i>	BAD00019.1	100	AAK49790.1	100
Sheep	<i>Ovis aries</i>	ABO09983.1	99	AAF78069.1	99
Cow	<i>Bos taurus</i>	BAE20190.1	91	Q401B2*	96
Zebu	<i>Bos indicus</i>	ACN65830.1	90	C0LQK0*	96
Yak	<i>Bos grunniens</i>	AAV67873.1	91	G3MCF0*	96
Buffalo	<i>Bubalus bubalis</i>	Q645Q8*	88	C0LQK8*	95
Horse	<i>Equus caballus</i>	AGN95439.1	91	AGN95439.1	98
Pig	<i>Sus scrofa</i>	ABR08657.1	92	CAB40844.1	98
Chicken	<i>Gallus gallus</i>	D1MJC0*	78	ACY72559.1	97

* UniProt; The RS goat was taken as the reference for Nigerian goat

The phylogenetic tree constructed for Nigerian goat breeds with the sequences of other selected animals' exon 1 *MSTN* gene using UPGMA is presented (Figure 1). The Nigerian goat breeds and the published goat clustered in the same clade supported over a node with 69% bootstrap value. The small ruminants comprising of published sheep, published goat and Nigerian goat breeds were supported on a node with 97% bootstrap value. The published horse and pig were found to cluster together on a clade at a node with 88% bootstrap value. The published cow, yak and Zebu clustered in one clade and supported over 96% at the node. Buffalo was found supported over a 96% node with the other large ruminants. The published chicken sequence is distantly related to these other animals at the exon 1 region of *MSTN* gene.

The Nigerian SH and RS goat breeds and the published goat sequences clustered over a node with 48% bootstrap value and these were supported on the same clade with WAD goat at a 40% bootstrap value over a phylogenetic tree using UPGMA at the exon 3 region (Figure 2). All the goat breed sequences and the published goat sequence with the published sheep sequence were supported on a node with 82% bootstrap value. The published horse and pig were found to cluster together on a clade at a node with 68% bootstrap value and these were supported on the same clade with the published chicken sequence at a node with 74% bootstrap value. The published cow, yak and Zebu clustered in one clade and supported over 67% bootstrap value at the node.

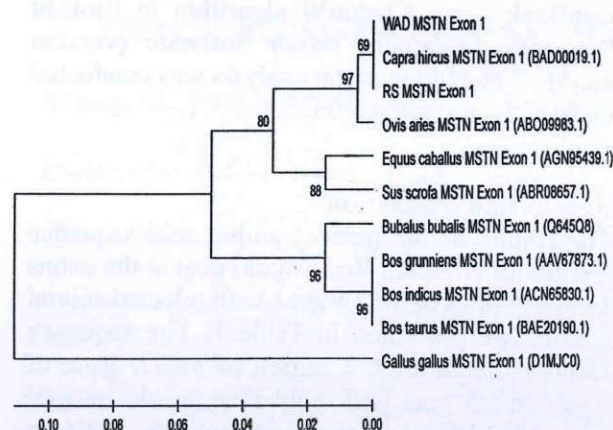


Figure 1. Phylogenetic tree constructed for Nigerian goat breeds and other exon 1 *MSTN* gene

Phylogeny analyses show sequences from within the same species having close relationships and clustering together. Macromolecules, in particular sequences, have gone beyond morphological and other organismal characters as the most accepted form of data for phylogenetic or cladistic analysis (Brinkman and Leipe, 2001). This study takes advantage of the utility of sequences for its molecular phylogenetic analysis. Comparative analysis of goat *MSTN* gene peptide sequence with that of sheep has been very closely related in cohort phylogeny. Phylogenetic closeness of goat with sheep as observed in this study has also been reported in *MSTN* gene (Li *et al.*, 2006) and other genes.

Chicken is genetically most distant to goat. Chicken was included in the phylogeny analysis as outgroup for possible outgroup rooting in the phylogeny reconstruction for *MSTN* gene exon 3 sequences. However, the outcome supports the underlying knowledge that *MSTN* gene is highly conserved among vertebrates. Outgroup rooting involves including a sequence that is known to be more divergent than the rest of the sequences in the analysis, and then making sure that it branches at the very base of the tree. Outgroup rooting is very reliable if solid prior information that permits choosing of a good outgroup is known (Guttman and Provart, 2014). Unfortunately this information is not known for many studies (Guttman and Provart, 2014).

Sheep and goat share the same cluster with 97% bootstrap value at the exon 1 region, and greater than 80% bootstrap value at the exon 3 region indicating that these two species have had a small number of mutations in *MSTN* gene (at these regions) since their break up in evolution. Similarly, horse and pig must have had small number of mutations at this gene from the time of

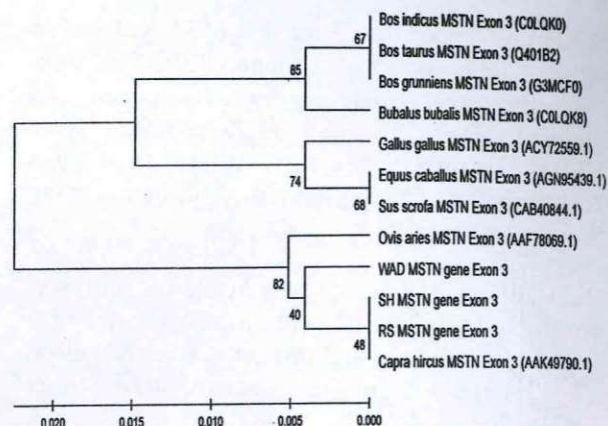


Figure 2. Phylogenetic tree constructed for Nigerian goat breeds and other exon 3 *MSTN* gene

their break up in evolution. This was supported in the work by Shah *et al.* (2006) where the researchers reported that sheep and cattle shared the same cluster with 96% homology to show the small amount of mutation that might have existed in the two breeds since their break up in evolution. The high homology of goat myostatin with selected animal species confirmed that vertebrate myostatin genes are highly conserved. Taking into consideration the existing high homology of *MSTN* among the vertebrates, there are grounds to suppose that the same correlation (Dybus *et al.*, 2013) will occur in goat. Shah *et al.* (2006) found high homology of more than 90% between camel myostatin with cattle, sheep and pig which confirmed that vertebrate myostatin genes are highly conserved. This implies that such high level of sequence conservation among all studied myostatin orthologs suggests the importance and conservation of its function in vertebrates.

Conclusion

The homology of *MSTN* in the evolution of Nigerian goat breeds and the selected species used for the study is highly conservative. This study providing a baseline framework on myostatin exons 1 and 3 regions in Nigerian goats and other selected animal species could be used as a target intervention to provide theoretical reference for further research.

References

- Alakilli, S.Y.M., Mahrous, K.F., Salem, L.M. and Ahmed, E.S. 2012. Genetic polymorphism of five genes associated with growth traits in goat. *Afri. J. Biotechnol* 11(82): 14738-14748.
- Baron, E. E., Lopes, M. S., Mendonça, D. and da Câmara Machado A. 2011. SNP

- identification and polymorphism analysis in exon 2 of the horse myostatin gene. *Anim Genet* doi:10.1111/j.1365-2052.2011.02229.x.
- Brinkman, F. S. L. and Leipe, D. D. 2001. Phylogenetic analysis. In: *Bioinformatics: A Practical Guide to the Analysis of Genes and Proteins, Second Edition* Eds: Andreas D. Baxevanis, B.F. Francis Ouellette, John Wiley & Sons, Inc., pp 323–358.
- Dehnavi, E., Azari, M. A., Hasani, S., Nassiry, M. R. Mohajer, M., Ahmadi, A. K., Shahmohamadi, L. and Yousefi, S. 2012. Polymorphism of myostatin gene in intron 1 and 2 and exon 3, and their associations with yearling weight, using PCR-RFLP and PCR-SSCP techniques in Zel Sheep. *Biotechnol Res Int.*, doi:10.1155/2012/472307.
- Dybus, A., Proskura, W.S., 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*). *Vet Med-Czech* 58 (1): 32–38.
- Grobet, L., Poncelet, D., Royo, L.J., Brouers, B. and Pirottin 1998. Molecular definition of an allelic series of mutations disrupting the myostatin function and causing double-muscling in cattle. *Mamm. Genome* 9:210-213.
- Guttman, D.S. and Provart, N.J. 2014. Lab 4 in *Bioinformatic Methods I*, University of Toronto, pp 1-19.
- Hickford, J.G.H., Forrest, R.H., Zhou, H., Fang, Q., Han, J., Frampton, C.M., Horrell, A.L. 2010. Polymorphisms in the ovine myostatin gene (*MSTN*) and their association with growth and carcass traits in New Zealand Romney sheep. *Anim Genet* 41: 64–72.
- Li, X.L., Wu, Z.L., Liu, Z.z., Gong, Y.F., Zhou, R.Y. and Zheng, G.R. 2006. SNP identification and analysis in part of intron 2 of goat *MSTN* gene and variation within and among species. *J. Hered* 97: 285-289 doi:10.1093/Jhered/Esj026.
- Shah, M. G., Qureshi, A. S., Reissmann, M. and Schwartz, H. J. 2006. Sequencing and sequence analysis of myostatin gene in the exon 1 of the camel (*Camelus dromedarius*). *Pakistan Vet J.* 26(4): 176-178.
- Singh, L.V., Tripathi, V., Sharma, R., Pandey, A.K., Maitra, A. and Mishra, B.P. 2012. Genetic polymorphism of *CAPN1* gene in Sirohi Goat. *Int. J. Meat Sci.* 2: 13-19.