Polymorphisms of Myostatin gene and its association with growth in two strains of Small East African and Blended goats of Tanzania

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Abstract

Despite the high demand for goat meat, the quantity of meat that is produced from the indigenous goats is low and insufficient to meet the demand. This is due to their small body size and inherent low genetic potential for growth coupled with poor management especially feeding. Improvement of goat productivity through selection takes long time to achieve and may be difficult for some traits. Information on polymorphisms in candidate genes for growth including myostatin gene could be used with pedigree information in marker assisted selection to get high genetic response more quickly. This study assessed polymorphisms of the intron 2 and exon 3 of the myostatin gene in Pare, Sonjo, Blended and Boer goats.

Only one singleton polymorphic site T298C was detected in the Boer goat population and all other goats were monomorphic. Two alleles, T and C were detected in Boer goats with frequencies of 0.98 and 0.02, respectively, and two genotypes TT and TC with frequency of 0.97 and 0.03, respectively. Allele T was fixed in the Blended, Pare and Sonjo populations. Blended goats were heavier at all stages of growth than Pare and Sonjo goats. However, due to lack of polymorphism in the three goat populations the association between the alleles of the myostatin gene and growth performance could not be confirmed. It can be concluded that there are variation in growth performance among the Blended, Pare and Sonjo goats but the variation could not be associated with the myostatin gene. Other genes for growth could be responsible for the observed variation.

Key words: *growth, local goats, myostatin, strain*

Introduction

Goats play an important role in the livelihoods and income generation of smallholder farmers in rural areas of Tanzania. The goats are mainly kept for meat production their meat is rank second to beef in terms of sales and consumption (Chenyambuga et al 2004). Goat meat in Tanzania is predominantly produced from the Small East African (SEA) goats which are raised in all ecological zones of the country. Other breeds that are used, though to a lesser extent alongside the SEA goats for meat production are the Boer and Blended goats. Pare and Sonjo goats belong to the Small East African goat breed which is well adapted and widely distributed in almost all ecological zones where they are used for meat production. Blended goats are the result of three-way crosses (55% Kamorai, 30% Boer and 15% indigenous), developed at Malya, Tanzania, which were stabilised in the late 1960s (Das 1989). They are dual purpose goats but are mainly kept for meat production because of their relatively higher growth rate and bigger mature size compared to the SEA goat strains (Das 1989). Since their development in the 1960s, Blended goats have been maintained mostly in Government farms and research stations where they are multiplied and distributed to farmers. The Boer goat is a meat purpose breed intensively selected for rapid growth (Malan 2000) and therefore, widely used in crossbreeding to improve goat productivity in different parts of the world. Despite the high growth advantage of the Blended goats and the excellent adaptation to the local conditions of the SEA goat strains, improvement through within breed selection has not been practised.

The demand for goat meat in urban areas has increased recently due to growth of tourism, expanding mining industries and establishment of international hotels in Tanzania (Kinunda-Rutashobya, 2003). Despite the high demand for goat meat, the biggest challenge remains with the quantity of meat that is produced from these animals. This is due to small body size and inherent low genetic potential for growth coupled with poor management especially feeding. Efforts to improve productivity of goats in Tanzania, like elsewhere in developing countries have always focused on crossbreeding rather than selection within the local stock. Selection for production traits has been practiced mostly in intensive production systems, essentially based on dairy recording schemes combined with artificial insemination (Montaldo and Manfredi 2002). In most developing countries, Tanzania included, goat breeding programs for local breeds under extensive systems are not common due to difficulties of setting up such a program in the marginal areas where goats are often raised (Lôbo *et al.,* 2010). Nevertheless, genetic improvement of locally adapted breeds is important to realizing sustainable production systems.

DNA technologies can be used to reliably realize intense and accurate selection and short generation intervals and to enable genetic improvement of locally adapted breeds to contribute to the required livestock development. Growth

traits of animals are regulated by many genes which are responsible for the economic value of the animal (Chen et al 2012). The genes are, therefore, important to consider when designing breeding programs and identification of such genes is critical for establishing marker-assisted selection (Li et al 2009). The current advances in molecular genetics have made possible the identification of individual genes or candidate genes with substantial effects on the traits of economic importance. The allelic and genotypic variation at the candidate genes of interest depicts the differences among breeds on genetic basis. This variation can be used together with traditional selection methods to accelerate the rate of change in economically important traits (Womack 2005). There are many published articles on different genes associated with meat-related traits in different goat breeds; among these genes is myostatin (*MSTN*). Myostatin is a member of the transforming growth factor- (TGF-) superfamily and it has been shown to repress muscular growth (Bellinge *et al*., 2005). Genetic variation at the *MSTN* gene has been reported among several goat breeds (Singh *et al*., 2014; Tay *et al*., 2004; Li *et al*., 2006) and shown in some breeds to affect body weight at different stages of growth (Zhang *et al*., 2013). However, polymorphism of the genes affecting growth traits in indigenous goats of Tanzania has not been studied. This study was, therefore, designed to investigate the polymorphisms of the *MSTN* gene and any possible association with growth performance of two SEA strains and Blended goats.

Materials and methods

Blood sampling and data collection

Blood samples were obtained from Pare (n = 44), Sonjo (n = 40), Blended (n = 31) and Boer goats (n = 29). All the animals were reared at West Kilimanjaro Research Centre except Boer goats used as a reference breed that were reared at Ngerengere Government farm. Blood samples were collected from the jugular vein of the goats using a 10 ml EDTA anticoagulant reagent sterile tube. Growth records were taken for Pare, Sonjo and Blended goats for four years from 2010 to 2013. The growth traits evaluated were; birth weight, weaning weight (at 16 weeks), and yearling weight (at 48 weeks).

DNA Amplification and Sequencing

DNA was isolated using standard commercial kit (Qiagen blood kit, Chartsworth, USA) according to the manufacturer's instructions. After quantification and dilution of the DNA, the region corresponding to the intron 2 and part of exon 3 of the goat *GDF*8 gene was amplified by polymerase chain reaction (PCR) using the following primer pairs; *MSTN*startF (5'-CCCTCCCTTTACTGTCATCC-3') and *MSTN*EstopR (5'- TCA TGA GCA CCC ACA GCG GTC -3'). Each 25 µL PCR reaction contained 50 ng of sample DNA, 0.4 µM of each primer, 1X PCR buffer (10 mMTris-HCl, pH 8.0, 50 mM KCl), 2.0 mM $MgCl_2$, 0.2 mM of each dNTP and 1 U of Taq DNA polymerase (Invitrogen). Amplification reactions were carried out in a thermal cycler (Applied BioSystems), with 5 min denaturation at 94˚C, 34 cycles of denaturation at 94˚C for 45 sec, annealing at 62˚C for 45 sec and extension at 72˚C for 1 min, and a final extension at 72˚C for 5 min. The PCR products were stored at 4°C and then detected by gel electrophoresis using 1% agarose gel. A total of 65 samples were successfully amplified; Pare (n = 11), Sonjo (n = 5), Blended (n = 20) and Boer (n = 29). The resulting 700 bp fragments were purified and sequenced with an automated sequencer (Applied Biosystems 3130).

Statistical analysis

The amplified fragment spanned a region from 1898 to 2276 bases including parts of intron 2 and exon 3. The resulting sequences were aligned using Mega V7 and the consensus sequences obtained were compared with the *MSTN* GenBank caprine sequences (DQ167575) and single nucleotide substitutions were identified. The SAS software (SAS Institute, Cary, NC, USA) was also used to analyse the differences in growth performance between goat populations. A Linear model was established with effects of population, sex and year of birth as shown below.

$Y_{ijk} = \mu + P_i + S_j + T_k + e_{ijkl}$ where Y_{ijk} = Phenotypic observations (Birth weight, weaning weight, yearling weight), µ = overall mean, P_i = effect of population (Pare, Sonjo and Boer), S_j = effect of sex (Male and Female), T_k = effect of year (2010, 2011, 2012 and 2013), e_{ijkl} = random error. It was not possible to assess the association between genotypes of the *MSTN* gene and growth performance as no polymorphisms was detected in the gene from the three populations on which growth data were recorded.

Results and discussion

Polymorphism of the *MSTN* locus in the goat strains/breed studied

Parts of intron 2 and exon 3 of goat*MSTN* gene (AY032689) were sequenced. The sequenced region from 65 goat samples was 631 bp long. Results show that the *MSTN* gene in the goat populations studied is highly conserved. Only one singleton polymorphic site T298C was detected in Boer goat population but in the rest of the individuals from other populations the *MSTN* gene was monomorphic. Table 1 shows the genotype and allele frequencies in the different populations. Two alleles, T and C were detected in Boer goats with frequencies of 0.98 and 0.02, respectively, and two genotypes TT and TC with frequency of 0.97 and 0.03, respectively. Allele T was fixed in the Blended, Pare and Sonjo populations.

Table 1. Allelic and Genotypic frequencies of the alleles and genotypes, respectively, detected at the *MSTN* locus in the four goat populations

Population	Allelic frequency		Genotypic frequency	
	T	C	TT	TC
Blended	1	0	1	0
Pare	1	0	1	0
Sonjo	1	0	1	0
Boer	0.98	0.02	0.97	0.03

Different studies have detected different number of polymorphic sites within the same regions of the *MSTN* gene. Seven polymorphic sites have been reported in intron 2 in Chinese goats (Li *et al*., 2006). Sequencing of all the exons of the *MSTN* gene revealed three nucleotide changes in Chinese (Tay et al 2004) and Indian goat breeds (Singh et al 2014). Digestion of *MSTN* fragments with restriction enzymes also found the presence of different genotypes in Saudi and Egyptian goat breeds (Alakilli et al 2012). However, Ahad et al (2016), reported no polymorphisms in exon 3 of the*MSTN* gene. Inconsistency in genetic polymorphisms of the *MSTN* gene has also been observed by many authors in different sheep breeds (Li et al 2006; Dehnavi et al 2012; Mahrous et al 2014 and Ahad et al 2016). According to Dehnavi et al (2012), the inconsistency in results from different studies may be attributed to breed differences, population and sampling size, mating strategies, geographical position effect, and frequency distribution of genetic variants. Lack of genetic variability for the *MSTN* gene in the Pare, Sonjo and Blended goats in this study may be due to the fact that the *MSTN* gene is conserved as these goat populations are closely related. Also the lack of variation at the *MSTN* locus may probably be due to mating strategies used. Animals used in the present study came from a research station where few sires are used for breeding thereby increasing the possibility of inbreeding to occur and fixing the few alleles that would otherwise be variable. Dehnavi et al (2012) pointed out that the use of few sires for breeding and small effective population size are the reason for high inbreeding level and consequently low heterozygosity.

Growth performance of the Small East African and Blended goats

Mean live body weights of Pare, Sonjo and Blended goats at birth, at weaning and at one year of age are presented in Table 2. Blended goats were the heaviest at all stages of growth as illustrated in figure 1. There was a significant difference in growth across years at all stages of growth; kids born in 2012 showed the best performance. The weight advantage of the Blended goats compared to the Pare and Sonjo is due to the fact that the former is a composite breed purposely developed and selected for fast growth and large mature size. Additionally, Blended goats were developed through crossbreeding that involved among other breeds, the Boer breed which has been intensively selected for fast growth and large mature size. Since their development as a breed, Blended goats have been used in different interventions by the government and goat producers to increase goat body size due to their high growth potential. They have been used in breeding programs by being backcrossed to Small East African goat strains in different production environments. Fast growth in mammals is determined by the increases of muscle cell growth and proliferation. Myostatin affects growth negatively by inhibiting differentiation of myoblasts and the proliferation of myogenic cells (Thomas et al 2000; Wiener et al 2009). Presence of different variants of the *MSTN* gene was hypothesized to be the cause of differences in growth of goat populations. Thus, one of the objectives of the present study was to identify possible genetic variations of the *MSTN* locus and evaluate their effect on growth of the studied goats. Lack of variation at the *MSTN* locus in Blended, Pare and Sonjo goat populations, despite the observed differences in their growth performance, suggests that *MSTN* locus is not the genetic basis for the observed phenotypic variation. Other loci related to growth hormone axis have been intensively analysed and showed to be associated with different growth parameters (Marcel Amills 2014). Analysis of other candidate gene could establish the genetic basis of the observed variation in growth performance of the studied goats.

Table 2. Least squares means for weight at different growth stages of three goat populations

Factor	Level	Birth weight	Weaning weight	Yearling weight
Breed	Blended	2.98 ± 0.11^{a}	10.50 ± 0.44^{a}	16.80 ± 0.57^{a}
	Pare	2.56 ± 0.07^{b}	9.31 ± 0.34^{b}	15.17 ± 0.38^{b}
	Sonjo	2.37 ± 0.09^{b}	8.67 ± 0.42^{b}	14.00 ± 0.45^{c}
Sex	Male	2.66 ± 0.08	9.72 ± 0.37	15.58 ± 0.40
	Female	2.61 ± 0.07	9.26 ± 0.31	15.06 ± 0.37
Year of birth	2010	2.43 ± 0.10^{a}	9.48 ± 0.30^{a}	14.88 ± 0.40^{a}
	2011	2.14 ± 0.11^{a}	8.56 ± 0.46^{b}	12.52 ± 0.79^{c}
	2012	3.35 ± 0.15^{b}	11.48 ± 0.61^{c}	16.44 ± 0.58^{b}
	2013	3.08 ± 0.08^{b}	9.73 ± 0.42^{a}	15.83 ± 0.54^{ab}

Values bearing different superscript letters down the columns differ significantly at $P \leq 0.05$

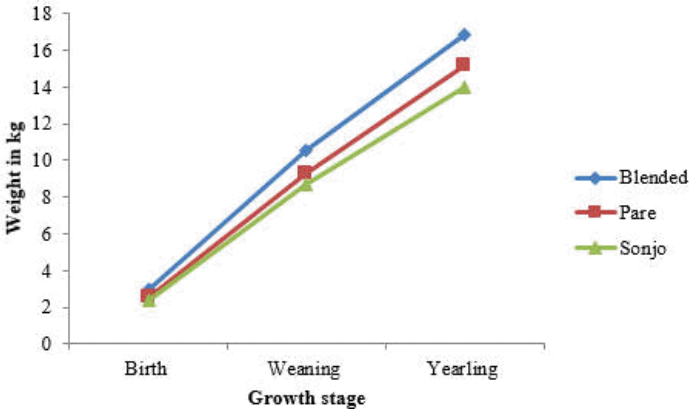

Figure 1. Growth performance of three goat strains at different stages of growth

Conclusions

- From the results of this study, parts of the intron 2 and exon 3 of the myostatin locus are monomorphic and, hence, highly conserved and, therefore, cannot be used as a biomarker for marker assisted selection in the studied goat breeds/strains.
- Blended goats are heavier at birth, weaning and one year of age than the Pare and Sonjo goats.
- Further studies targeting the whole *MSTN* locus and using larger sample size and animals from different production environments should be carried ou

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Conflict of interest

The authors have no conflict of interest to declare.

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