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Morphometric variation and muscle growth genes polymorphism between two indigenous sheep populations

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ABSTRACT: Ethiopia owns significant sheep genetic resources, but their potential for meat production remains underutilized. Hence, there is a need to characterize their morphometric traits and muscle growth gene polymorphisms of sheep populations. The study aimed to identify the morphometric variation and genetic polymorphism of the Callipyge (CLPG) and Myostatin (MSTN) genes in two sheep populations. Sheep from the North and West Shewa zones of Oromia National Regional State were purposively selected, and morphometric data (body weight and linear body measurements) were collected from 540 sheep (270 from each zone). Blood samples were collected from 180 sheep (90 from each zone) using vacutainer tubes with EDTA for genotyping at the CLPG and MSTN loci. DNA extraction and quality assessment were carried out using salting out procedure and gel electrophoresis, respectively. Polymerase chain reaction amplification was performed with gene specific primers. The amplified products were digested with restriction enzymes and visualized using gel electrophoresis. Morphometric data were analyzed using SPSS, while Popegen32 software was used for analysis of genotype data. Significant variation ($p < 0.05$) was observed in body weight and linear body measurements and morphometric indices between the two sheep populations. West Shewa sheep showed higher values in most trait and indices considered. Two alleles (A and G) and three genotypes (AA, AG, GG) were detected at the CLPG locus, and two alleles (M and m) with three genotypes (MM, Mm, mm) were identified at the MSTN locus in both populations. In North Shewa sheep, allele frequencies for A and G were equal (0.5), whereas allele G (0.57) was more frequent than allele A (0.43) in West Shewa. For the MSTN gene, the allele frequency of m (0.55) was higher than M (0.45) in North Shewa, while allele M (0.6) was more frequent than allele m (0.4) in West Shewa. Both genes were polymorphic in both populations, with expected heterozygosity and the number of alleles being comparable. The study revealed morphometric variation and polymorphism at the CLPG and MSTN loci in two sheep populations. Further studies with larger sample sizes and different ecotype of sheep populations are needed to validate the current findings and explore the potential of these genes as genetic markers for meat production.

Keywords/ phrases: Callipyge, Characterization, Genetic diversity, Myostatin and PCR-RFLP

INTRODUCTION

Myostatin and Callipyge are the most well-known major genes which are related with growth and meat quality traits in domestic animals. In sheep, mutations in the Myostatin and callipyge had an important role in muscular development and meat quality (Boman et al., 2009). As a consequence, the Myostatin and Callipyge genes are primarily responsible for muscle development and could be potential candidate genes for animal muscle growth.

Myostatin gene, also named as growing and differentiation factor-8 (GDF-8) gene, is coding for converting growth factor beta (TGF-b) super-family. This gene was physically mapped to sheep chromosome 2 (Archibald et al., 2010). It is also a powerful candidate gene for growth and development of domestic animals due to its key function in muscularity, and its potential applications in sheep farming (Shau et al., 2017). Callipyge is also another important gene that has been localized in the telomeric region on ovine chromosome 18 (Nanekarani et al., 2014). The best documented mutation for muscle development in sheep is callipyge, which causes a postnatal muscle hypertrophy that is localized to the pelvic limbs and loin (Cockett et al., 2005).

Genetic polymorphism of both genes has been reported in different breeds of sheep (Dehnavi et al., 2012; Iroanya et al., 2021; Bayraktar and Shoshin, 2022). Natural mutations exist in sheep

that affect muscle growth and development, and the exploitation of these mutations in breeding strategies has the potential to significantly improve lamb-meat (Nanekarani et al., 2014). Most of the traits related with meat/mutton production and meat quality would be evaluated after the animal is matured or slaughtered. So that efficient tools to facilitate selection on live animal and /or at early age would have a paramount importance for getting and sustainable utilization of breeding rams and ewe. Characterizing Mysotatin and Callipyge gene polymorphism will pave the way for identification of efficient marker and selection of sheep for quality meat production.

Ethiopia is home to a vast livestock resource, with 70 million cattle, 42.9 million sheep, 52.5 million goats, and 57 million poultry (CSA, 2021). Despite the large population of sheep, the productivity of Ethiopia's smallholder production system is limited due to Biological, Environmental, and Socioeconomic factors (Mohammed, 2015; Osman et al., 2021). Both export and domestic markets for mutton and live animal is increasing and it has been largely driven by human population growth, increasing income and expansion of urbanization. Besides, the strategic location of Ethiopia in relation to Middle East is also an opportunity to export meat (largely from sheep) and live animals. However, the productivity of indigenous sheep is currently too low, 10 kg off take to meet the current strong demand (Ameha Sebesbe et al., 2011) which is lower than the neighboring African countries sheep such as Sudan (12 kg); Kenya (13 kg) and Djibouti (14 kg) (Firew Tegegne and Getent Assefa, 2010). Hence, there is a need to improve sheep productivity to meet the protein demand by the ever increasing human population and to ensure conservation and sustainable utilization of the genetic resources. Improving meat production is in line with the Ethiopia 2030 agenda: The Pathway to Prosperity Ten Years Perspective Development Plan and in particular with increasing meat production from 295 thousand tons to 1.7 million tons (PDC, 2021). Improving the productivity of sheep will improve the livelihoods of producers and alleviate poverty among the rural poor dwellers and improve the country's foreign currency earning (Zewdu Eda et al., 2012).

However, there is limited information in both Mysotatin and Callipyge gene polymorphism in Ethiopian sheep populations. Most of the previous initiatives on molecular characterizations of Ethiopian sheep focused on diversity analysis and breed classification (Solomon Gizaw et al., 2007; Zewdu Edea et al., 2017; Helen Nigussie et al., 2019). Understanding the variation of economically important morphometric traits and genetic polymorphism of Mysotatin and Callipyge gene in sheep will be used as baseline information to design selection tools for sustainable genetic improvement and conservation. This is therefore; the current study was conducted to identify morphological variation and genetic polymorphism of Myostatin and Callipyge genes between sheep population in West and North Shewa zone of Oromia National Regional Sate.

MATERIAL AND METHODS

Description of the study area

The study was conducted in North (Debre Libanos and Wuchale districts) and West (Dandi and Ejere) Shewa Zones, Oromia National Regional State.

Debre Libanos district is located at 85 km north of the capital city, Addis Ababa in 38^{o} 58' 33" E longitude and 9^{o} 63' 75''N latitude with altitude ranging from 1500 to 2700 meters above sea level (m.a.s.l.). Its maximum and minimum annual temperature is 23^{0}C and 15^{0}C, respectively. The wet season lasts from May to September, and the dry season lasts from October to April. It is consists of 60% dega (highland), 30% Weinadega (mid-highland) and 10% kola (lowland). The land use pattern consists of 25,472 ha covered with annual crops, 2547 ha grazing land, 833 ha forest and manmade forestlands and 2276 ha for other purposes. The total livestock population in the district is estimated at 141,056 heads, out of which 63,786 (45.22%) are cattle, 50,000 (35.45%) sheep, 4800 (3.40%) goats, 12,700 (9.0%) equines. The population of chicken is estimated to be 37,000 (DLARDO, 2010).

Wuchale district is located at 75 km north of the capital city, Addis Ababa in 38^{0} 47'E longitude and 9^{0}54'N latitude with minimum of 3^{0}C and maximum temperature of 25^{0}C, respectively. The major wet season in Wuchale district lasts from May to September, with the dry season lasting from October to April. Agro-ecologically, dega (highland), weinadega (mid-highland) and kola (lowland) accounted for about 85.54%, 11.06% and

3.4% were Wuchale district's entire land area, respectively. Land use pattern is made up of 21,365 ha covered with annual crops, 23,023 ha grazing land, 747 ha forest and manmade forestlands and 3745 ha for other purposes. The total livestock population in the district is estimated at 289, 909 heads, out of which 123,256 (42.51%) are cattle, 128,953 (44.48%) sheep, 3,456 (1.19%) goats and 34,244(11.81%) equines. The Population of chicken is about 54,081(WARDO, 2022).

Dandi district is located at a distance of 78 km west of the capital city, Addis Ababa and 35 km east of Ambo, the town of the West Shewa zone. The district receives an average annual rainfall ranging from about 750mm to 1,170mm. The minimum and maximum daily temperatures of the area were 9.3°C and 23.8°C, respectively. The district is located at an elevation of 2000 to 3288 metres above sea level (m.a.s.l.) and has two agro-ecological zones: highland (44%), and midland (56%). Cattle (346,124), sheep (192,433), goats (28290), equines (57,403), and poultry (171656) are among the animal species found in the district. There are 192,433 sheep in the livestock population, and the district has a strong potential for both sheep and cattle production (DWLFDO, 2022).

Ejere district is located 40 kilometres west of the capital city, Addis Ababa, The average annual rainfall in the district ranges between 900 and 1,200 mm. The minimum and maximum daily temperatures of the area are 9°C and 28°C, respectively. The district is located between 2060 and 3,185 m.a.s.l and has two agro-ecological zones: 45% highland (>2300 m.a.s.l.) and 55% midland (1500 to 2300 m.a.s.l.). Cattle (114009), sheep (62,949), poultry (106,412), equines (32,392), and goats (27,373) are among the animal species found in the district. Crop-livestock mixed farming system is a dominant production system in both zones of the study sites (EWLFDO, 2022).

Sheep populations and Sampling strategy

Reconnaissance survey was conducted to select the study areas and sampling site. The sampling sites were selected purposively based on their potential for sheep production, road accessibility, and farmers' willingness to participate in the study in consultation with agricultural experts both at the zonal and district level. Accordingly, two districts were selected from each zone. Three rural kebeles were selected from each district. Generally, a total of twelve rural kebeles were selected from the four districts of North and West Shewa zones. A total of 240 households, 60 from each district, were selected randomly (Table 1). A total of 540 individual sheep (270 from each zone) were selected for body weight and body linear measurement. From each sampling site, 45 sheep (two to three animals per flock) were sampled. Only ewes having two or more than two Pair of Permanent Incisor (PPI) were considered in this study due to the shortage of matured ram in most of the study areas (Table 1).

Table 1. Number of sampling sites, sheep used for body measurements data and blood samples collection in the study areas.

Zones (N=2)	Districts (N=4)	Number of Households (N=240)	Number of sheep for body measurement data collection (N=540)	Number of sheep for blood samples collection (N=180)
North Shewa	Muketuri	60	270	45
	D/libanose	60		45
West Shewa	Dandi	60	270	45
	Ejere	60		45
Total		**240**	**540**	**180**

Morphometric traits data collection

Data on morphometric traits were collected from all 540 sheep. Accordingly, eleven morphometric traits (body weight, body length, height at withers, rump height, chest girth, chest width, Chest depth, Ear length, cannon bone circumferences, Tail length and tail width) were measured from all individuals. All measurements except body weight, height at wither and rump

height were taken using a measuring tape calibrated in centimeters (cm) after restraining and holding the sheep on flat and hard grounds. The Body weight (kilogram) was measured using suspended spring balance with 50kg capacity. Height at wither and rump length measurements were taken using measuring stick (centimeter) as described in FAO descriptor list (FAO, 2012).

Blood samples collection and DNA extraction

Ethical permission for collection of blood samples was taken from the agriculture office of the research area and blood samples were taken from selected sheep using professional veterinarian. Blood samples were collected from 180 sheep (90 from each zone) (Table 1). Five milliliters of blood were drawn from the jugular vein using vacutainer tubes containing Ethylene Diamine Tetra Acetic Acid (EDTA) as an anticoagulant and kept refrigerated until further analysis. Genomic DNA was extracted from whole blood using salting out procedure (Bruford et al., 1992). The quality of the DNA was checked on 0.6% agarose gel electrophoresis. The gel was run using a 1X TAE buffer and stained with Ethidium Bromide (1.75µg/ml) with 1kb DNA ladder and loading dye for 45 minutes. The genomic DNA was visualized as exposure to UV light. The purity and concentration of DNA was determined by taking spectrometric readings using NanoDrop. One hundred twenty (120) samples having optical density ratio (OD260/OD280) between 1.8 - 2.0 and 50 ng concentration were used for Polymerase Chain Reaction (PCR) and the rest 60 samples were not eligible for PCR due to their poor DNA quality.

PCR amplification

The Exon 3 part of the 337 bp MSTN and 214bp CLPG gene fragment was amplified using primer sequence, F: CCG GAG AGA CTT TGG GCT TGA and R: TCA TGA GCA CCC ACA GCG GTC designed by (Dehnavi et al., 2012) and F: GGA ATC ATC GTG TCC TGG TC and R: CCA GCA GGA TAC TCC GTG TC (Qanbarie et al., 2007), respectively. Polymerase chain reactions (PCR) were carried out in a total reaction mixture of 20 µl reaction volume containing 5 µl master mix (dNTP, Taq polymerase, PCR buffer and $Mgcl_2$), 0.5 µl each of forward and reverse primers (10 pmol/µl), 1 µl template DNA (50 ng/µl) and 13 µl of nuclease-free water. The amplification reaction was carried out with a program of 5 min denaturation at 95°C for 35sec followed by 30 cycles annealing at 60°C for 30 sec and with a final extension for 5 min at 72°C.

Restriction fragment length polymorphism (PCR-RFLP) assay

Restriction digestion of PCR products of Callipyge and Myostatin were carried out in 15 µl reaction mix comprising 8 µl nuclease-free water, 5 µl PCR Product, 1.5 µl 10XNE buffer and 0.5 µl restriction enzymes (*AvaII* and *HaeIII,* respectively). The PCR products were incubated at 37°C for 2 hrs. The digestion products were checked at 2% Agarose gel with 50 bp marker ladder and treated with luminescent dye to make the DNA visible and visualized using a Bio-Rad gel documentation system.

Data Analysis

Morphometric traits variation

The morphometric indices were used to assess the balance between aesthetics and production potential of an animal. Index was calculated for Body, Dactyl thorax, Depth, Foreleg length Height, Length. Height slope and Body ratio were also calculated according to the formula adapted from (Banerje, 2017) as indicated in Annex Table 1.

The mean and standard Error (SE) of the morphometric traits and morphometric indices were estimated using GLM procedure of SPSS, ver. 26 (SPSS, 2019). One-way ANOVA was used to compare means of the morphological traits and to analyze the effects of the fixed factor (sheep type or population) on the response variable. The following general linear model was used for the analysis of morphometric traits.

$Yi= \mu+Pi + ei$, where,

Yi is response of the observed variables

µ is population mean

Pi is the fixed effect of the i^{th} sheep population (i= North and West Shewa)

ei is the random error

Genetic polymorphism analysis

PCR-RFLP data analysis were done using Popgene32 Version 1.31 to determine genetic diversity parameters i.e., genotype frequency, allele frequency, observed heterozygosity (Ho), expected heterozygosity (He), percentage of polymorphic loci (P%), effective number of allele

(Ne), Shannon's Information index (I) and Hardy–Weinberg equilibrium (HWE); X^2 test (Yeh et al., 1999).

RESULT

Morphometric variation between North and West Shewa sheep populations

The analysis of variance of the morphometric traits in studied sheep populations are shown in Table 2. Significant variation ($p < 0.05$) was observed between sheep populations in all traits considered. Most of the height (withers and rump height), conformation (chest girth and chest width), and body weight and body length were higher in West than North Shewa sheep populations. However, rump and tail length were higher in North than West Shewa sheep (Table 2).

Table 2. Mean squares (mean± SE) of morphometric traits in North and West Shewa sheep populations.

Traits	Sheep Populations			Sign
	West Shewa (N=270)	North Shewa (N=270)	Total (N=540)	
Body weight	26 ± 0.36	30 ± 0.19	28 ± 0.22	*
Body length	57 ± 0.36	61 ± 0.36	59 ± 0.27	*
Withers height	64 ± 0.27	65 ± 0.28	65 ± 0.20	*
Chest girth	67 ± 0.39	72 ± 0.31	69 ± 0.27	*
Chest width	18 ± 0.18	19 ± 0.14	18 ± 0.11	*
Chest depth	28 ± 0.21	35 ± 0.21	32 ± 0.21	*
Rump height	67 ± 0.27	65 ± 0.28	66 ± 0.20	*
Cannon bone circumference	6 ± 0.04	11 ± 0.06	9 ± 0.10	*
Ear length	9 ± 0.13	13 ± 0.26	11 ± 0.16	*
Tail length	32 ± 0.40	28 ± 0.26	30 ± 0.25	*
Tail width	11 ± 0.15	21 ± 0.22	16 ± 0.25	*

**** Significant at p<0.05***

Indices (Mean ± SE) of the morphometric traits in North and West Shewa sheep populations are shown in Table 3. A significant variation ($p< 0.05$) was observed in most indices except body index among sheep populations in North and West Shewa. The higher value in Dactyl–thoracic index, height slope, depth and length indices were observed in West Shewa sheep, while higher value in foreleg length and height index were observed North Shewa sheep. Height slope index indicated that West Shewa sheep is relatively sloppier than the North Shewa population. The length index in terms of height showed that West Shewa sheep is longer bodied than North Shewa (Table 3).

Table 3. Morphometric indices (Mean ± SE) of sheep in North and West Shewa.

Indices	Sheep populations			Sign.
	West Shewa (N=270)	North Shewa (N=270)	Total (N=540)	
Body index	85.59 ± 0.75	85.39 ± 0.79	85.49 ± 0.54	ns
Dactyl–thoracic index	15.19 ± 0.12	9.55 ± 0.90	12.37 ± 0.14	*
Depth index	0.53 ± 0.00	0.43 ± 0.00	0.48 ± 0.00	*
Foreleg length	30.41 ± 0.35	36.19 ± 0.27	33.30 ± 0.25	*
Height index	108.16 ± 0.73	115.53 ± 1.36	111.84 ± 0.79	*
Height slope	-0.14 ± 0.18	-2.35 ± 0.30	-1.24 ± 0.16	*
Length index	0.93 ± 0.01	0.88 ± 0.01	0.90 ± 0.01	*
Over increase index	100.21 ± 0.16	103.9 ± 0.52	102.10 ± 0.29	*
RLI	119.79±1.39	108.38 ±0.76	114.09 ±0.83	*
LPI	103.99±0.52	100.24 ±0.16	102.12 ±0.29	*
RDTI	115.53±1.37	108.12 ±0.74	111.83 ±0.79	*
RTI	43.91±0.33	53.70 ±0.42	48.80 ±0.34	*
PRI	9.76±0.07	16.62 ±0.12	13.19 ±0.16	*

*Longitudinal pelvic Index (LPI); RDI= Relative depth of thorax; RTI=Relative thickness of cannon bone; PRI=Proportionality Index; ns=not significant, * p<0.05*

Callipyge and Myostatin gene polymorphisms between two indigenous sheep populations

From 120 DNA samples subjected to PCR amplification only 60 and 90 samples from Callipyge and Myostatin primers were amplified, respectively. The PCR products were visualized at 1.75% agarose gel using Bio-Rad gel documentation system. Clear image of 214bp and 337bp fragments were observed for Callipyge and Myostatin genes, respectively. 100 base pair marker ladder was used for allele size determination (Figure 1 and 2).

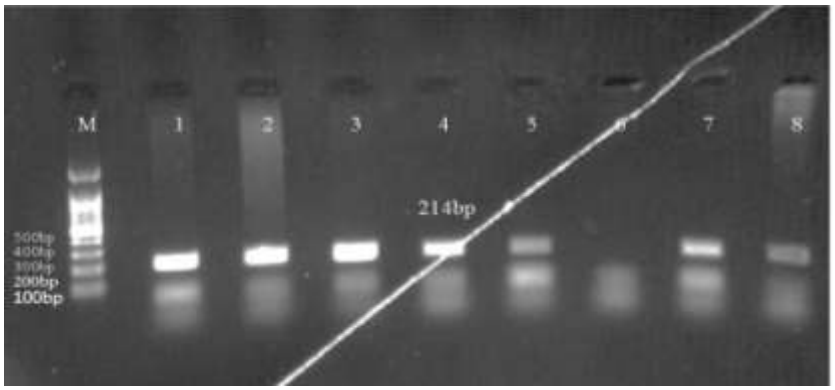

Figure 1. Amplification of CLPG gene fragment in 2% agarose gel. Lane ML= marker ladder (100bp), Lane 2 to 8 is the 214 bp amplicon of callipyge gene, and lane 6 is the negative control (-Ve).

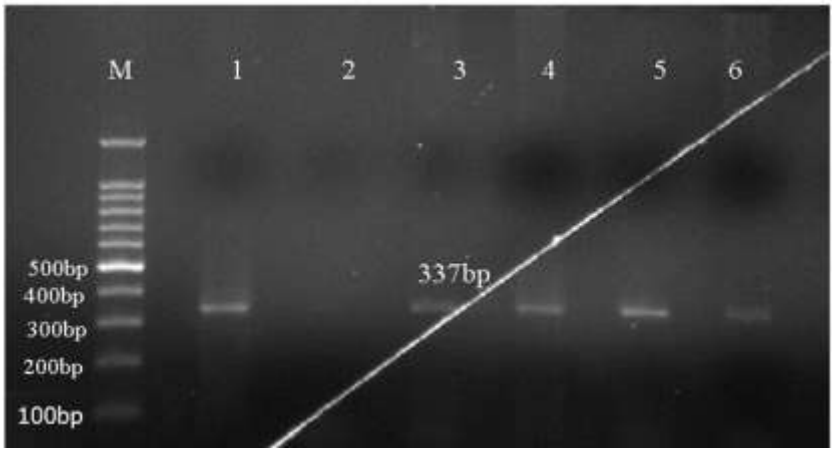

Figure 2. PCR amplification of MSTN exon 3 fragments in 2% agarose gel. ML= Marker ladder, Lane 1, 3 to 6 is the 337 bp amplicon of MSTN exon 3 and lane 2 is the negative control without genomic DNA.

Restriction Fragment Length Polymorphism of Callipyge gene

Restriction digestion of the PCR products for the CLPG gene which was carried out using *AvaII* restriction enzymes in indigenous sheep populations is presented in Figure 3. However, out of 60 amplicon of Callipyge gene subjected to restriction digestion, only 33 of them were successfully digested and visualized. Two alleles, A and G, and three genotypes, AA (137 and 77 bp), AG (214, 137 and 77 bp) and GG (undigested 214bp) were identified (Figure 3).

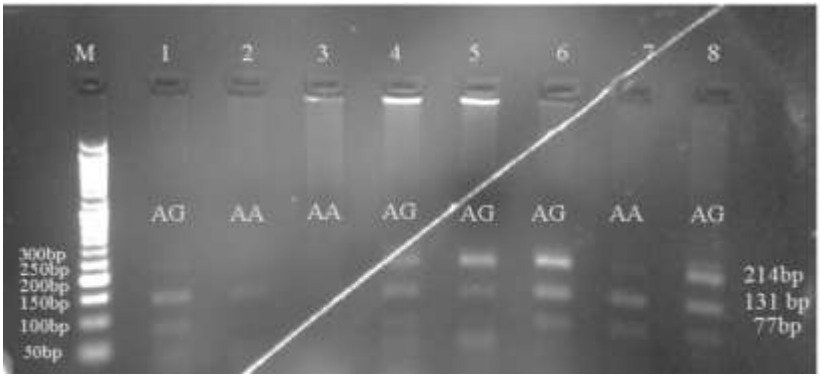

Figure 3. Restriction enzyme patterns of CLPG gene amplicons were digested with *AvaII* restriction endonuclease enzymes; M= Marker Ladder (50bp)

Allelic and genotypic frequencies at Callipyge locus

Allele and genotype frequencies of Callipyge locus in sampled populations are shown in Table 4. Both North and West Shewa sheep populations showed A and G Allele with different allelic frequencies. Both A and G frequencies were equal (0.5) at North Shewa sheep while allele G (0.57) was more frequent than allele A (0.43) in West Shewa sheep population. Genotypic frequency of AG was more frequent than AA and GG in both populations. However, AG is higher in North Shewa (0.80) than West Shewa (0.73) sheep population (Table 4). There was a significant difference in genotypic frequency of CLGP locus in North Shewa sheep.

Table 4. Allelic, Genotypic frequencies and Chi-square test for Hardy-Weinberg equilibrium of CLPG locus in two indigenous sheep populations

Populations	Alleles	Allele frequency	Genotypes	Genotype frequency	Genotype observed	Genotype Expected	χ^2
North Shewa	A	0.50	AA	0.10	2	4.50	5.56*
	G	0.50	AG	0.80	14	9.00	
			GG	0.10	2	4.50	
West Shewa	A	0.43	AA	0.07	1	2.80	3.65^{ns}
	G	0.57	AG	0.73	11	7.40	
			GG	0.20	3	4.80	

ns=not significant, * $p<0.05$

Genetic polymorphism within and between sheep populations at Callipyge locus

The levels of genetic variation within population are given in Table 5. The effective number of alleles provides information on genetic diversity within the population, which was 2.00 in north Shewa and 1.97 in West Shewa. Callipyge locus was polymorphic in both North and West Shewa sheep populations. The same trend was also observed in Shannon index value (0.69 and 0.64) and Expected heterozygosity value (0.50 and 0.49) in both North and West Shewa sheep populations, respectively. However, the Observed Heterozygosity was higher than the Expected Heterozygosity in both breeds. A positive correlation was observed between the effective numbers of alleles per locus and mean Expected Heterozygosity (Table 5).

Table 5. Genetic polymorphism measure (Mean±SE) of CLPG locus of North and West Shewa sheep populations.

Genetic polymorphism indices	Sheep populations		Overall
	North Shewa	West Shewa	
No. of Effective Alleles	2.00±0.32	1.97±0.02	1.98±0.02
Proportion of Polymorphic Loci	100±0.02	100±0.02	100±0.02
Shannon's Information index	0.69±0.13	0.68±0.01	0.69±0.02
Observed Heterozygosity	0.78±0.23	0.73±0.07	0.76±0.02
Expected Heterozygosity	0.50±0.12	0.49±0.01	0.49±0.02

Restriction Fragment Length Polymorphism of Myostatin gene

Restriction digestion of the PCR products for the Myostatin gene using *Hae III* restriction enzymes in indigenous sheep populations is presented in Figure 4. Out of 90 PCR products of Myostatin gene subjected to restriction digestion, only 39 of them were successfully digested and visualized at 2% agarose gel from both sheep populations. Two alleles, M and m, and three genotypes, MM (undigested 337bp), Mm (337,131,123 and 83bp) and mm (131, 123 and 83bp) were identified through restriction digestion of the PCR product of the MSTN locus (Figure 4).

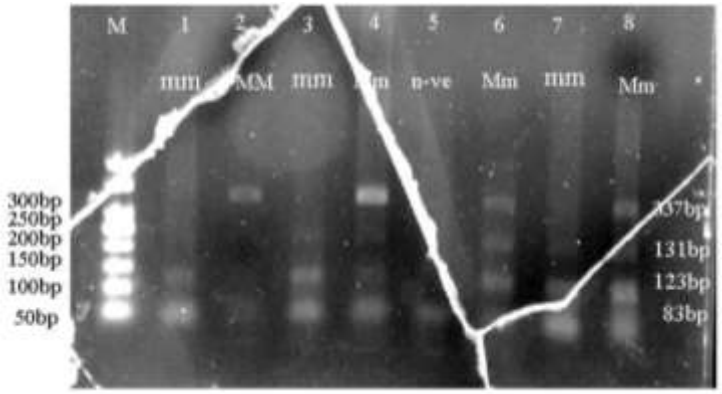

Figure 4. Restriction enzyme patterns of MSTN gene amplicon digested with *HaeIII* restriction endonuclease enzymes; M= marker ladder (50bp)

Allele and genotypic frequencies of Myostatin locus

Allele and genotype frequencies of Myostatin locus in North and West Shewa sheep populations are shown in Table 6. Both sheep populations showed M and m allele with different frequencies. There was a variation in allele and genotype frequencies. Allele m was more frequent (0.55) than M (0.45) in North Shewa sheep while allele M was more frequent (0.6) than m (0.4) allele in West Shewa population. Genotypic frequency of Mm was higher followed by mm and MM in North Shewa. However, the genotypic frequency of MM and Mm is equal (0.4) in West Shewa sheep while it was 0.2 in mm allele (Table 6). There was no significant difference in genotypic frequency at myostatin locus between the sheep populations.

Table 6. Allelic, Genotypic frequency and Chi-square test for Hardy-Weinberg equilibrium of MSTN locus in two indigenous sheep populations

Populations	Alleles	Allele frequency	Genotypes	Genotype frequency	Genotype observed	Genotype Expected	χ^2 df=1
North Shewa	M	0.45	MM	0.16	3	3.80	0.456^{ns}
	m	0.55	Mm	0.58	11	9.40	
			mm	0.26	5	5.80	
West Shewa	M	0.60	MM	0.40	8	7.20	0.456^{ns}
	m	0.40	Mm	0.40	8	9.60	
			mm	0.20	4	3.20	

ns=not significant

Genetic polymorphism within sheep populations at MSTN locus

The levels of genetic variability between and within a population are given in Table 7. The effective number of alleles provides information on genetic diversity within the population, which was 1.98 in North Shewa and 1.92 in West Shewa. Expected heterozygosity estimates within the MSTN loci showed that both sheep populations have similar expected heterozygosity. The expected heterozygosity was slightly lower than the observed heterozygosity. A positive correlation was observed between the effective numbers of alleles per locus and mean expected heterozygosity (Table 7). The Shannon Index value was 0.688 and 0.673 in North and West Shewa sheep, respectively (Table 7).

Table 7. Genetic polymorphism measure (Mean±SE) of MSTN Locus of North and West Shewa sheep populations.

Genetic polymorphism indices	Sheep populations		Overall
	North Shewa	West Shewa	
No. of Effective Alleles	1.98±0.32	1.92±0.02	1.95±0.02
Proportion of Polymorphic Loci	100±0.02	100±0.02	100±0.02
Shannon's Information index	0.69±0.13	0.67±0.01	0.68±0.02
Observed Heterozygosity	0.58±0.23	0.40±0.07	0.76±0.02
Expected Heterozygosity	0.49±0.12	0.48±0.01	0.49±0.02

DISCUSSIONS

Morphometric variation between North and West Shewa sheep populations

Information on morphometric traits is required for long-term breed improvement, genetic resources utilization and conservation. Morphometric traits can describe the characteristics of a breed (Solomon Gizaw et al., 2011). The present study revealed that there were major variations between sheep populations in North and West Shewa in terms of body weight (BW), chest girth (CG), body length (BL), ear length (EL), tail length (TL), and chest depth. West Shewa sheep showed better live weight and most of the linear body measurements than North Shewa sheep except Rump length and Tail length. This is in line with the report of (Bosenu Abera et al., 2014; Ashebir Worku, 2019) who reported that there were significant differences in the body weight, chest girth, and body length of sheep populations in North Shewa Wuchale and D/Libanose and West Shewa, West Arsi Zone of Oromia National Regional State, respectively.

Considering body length in relation to wither height, both populations are shown to be taller and the chest girth of both populations was proportional to their size. However, the higher body index, dactyl–thoracic index, depth index, height slope, body ratio, and length index showed that the West Shewa sheep are larger, have a longer body, and have a more upright body posture than the North Shewa sheep. According to Banerjee (2017) and Salako (2006) sheep having better body index, dactyl–thoracic index, depth index, height slope, body ratio, and length index would have a potential for better meat production.

The indices will be used as a tool to select a potential sheep for breeding purpose. The larger body size and better index value of sheep in West Shewa might be due to better husbandry practice and feed availability in the area compared to North Shewa. Husbandry practice, feed and water availability affect the performance of the animals (Knapik et al., 2017).

Genetic polymorphism at Callipyge locus

The callipyge lambs exhibit some desirable commercially important characteristics and meat quality properties, such as a higher percentage of meat yield, a larger loin part, leaner meat, and higher limbs (Jackson et al., 1997b). In the present study, the genetic polymorphism of callipyge gene was identified both in two indigenous sheep populations at North and West Shewa. The genetic polymorphism identified in the current study is in line with the report of (Jackson et al., 1997a ; Shah et al., 2018; Esen et al., 2022) who reported the genetic polymorphism of callipyge gene in various sheep breeds such as Dorset, Ramboulee, Hampshire, Thalli, kvrck, Karacabey Merino, Ramlç, German Black-Head Mutton Kvrck, and Hampshire Down Merino. It has also been reported that Callipyge gene mutation and expression can lead to a hypertrophy of the muscles in sheep contributing by increasing in muscles weight (the pelvic limbs and loin lion eye zones) (Cockett et al., 1996). The Callipyge gene offers a higher leg score and dressing percentage for Callipyge carcasses which are considered as a desirable characteristic in meat traits (Duckett et al., 1998; Koohmaraie et al., 1995). On the contrary, monomorphism of Callipyge gene was reported in different sheep breeds of Bulgaria (Bozhilova-Sakova et al., 2020), in Najdi, and Harri sheep population of Saudi Arabia (Alakilli, 2015) and in Edilbay, Volgograd, and Kalmyk sheep of the Russian Federation (Gorlov et al., 2019). Higher hertozygosity were observed in both populations, though relatively higher value was observed in North Shewa sheep than West Shewa which is consistent with the report of (Tolee et al., 2021) who reported higher frequency of AA (61%) genotypes in Iraq sheep. Higher Hertozygosity and Shannon Index value in both populations suggest that the genetic diversity is relatively high within both sheep populations.

Genetic polymorphism at Myostatin locus

Mutations within the Myostatin gene led to muscle enlargement allele in the double muscle breeds (Mosher et al., 2007). Therefore, harnessing myostatin gene polymorphism in meat animals will create an opportunity to select better animals for breeding and marker-assisted selection (Dehnavi et al., 2012). The findings of the current result in myostatin genotypic frequency are in line with the findings of Saygili and Ozdemir (2023) who reported that the Myostatin gene polymorphism in indigenous Morkaraman sheep with frequencies of 11.1%, 62.6%, and 26.3%, for MM, Mm, and mm respectively. But, it was lower than the report of Jamshidi et al. (2014), who revealed the existence of two MSTN alleles with the frequencies of 0.97 and 0.03 for m and M, respectively, in Mehraban's sheep. Similarly, Soufy et al. (2009) identified genetic variation in exon 3 of MSTN in Sanjabi sheep, reported two alleles, m and M, with frequencies of 0.97 and 0.03 and three genotypes, mm, Mm, and MM with frequencies of 0.97, 0.01, and 0.02, respectively. On the other hand, Myostatin gene monomorphism was reported in different sheep breeds in Romania (Lazăr et al., 2016), in Iran (Khederzadeh et el., 2016; Azari et al., 2012). The variation might be due to difference in breed and absence of mutation in these sheep breeds. Myostatin gene frequencies were in Hardy Winberg equilibrium in both sheep populations which are in agreement with the findings of (Bayraktar, 2020; Lazăr et al., 2016) who reported that Sanjabi sheep were in Hardy Winberg equilibrium. It implies that there is no selection of sheep for this particular trait in the study areas.

CONCLUSIONS

The present study revealed that there were a significant variations between the sheep populations in North and West Shewa in terms of body weight (BW), chest girth (CG), body length (BL), ear length (EL), tail length (TL), and chest depth. West Shewa sheep showed better live weight and linear body measurements than North Shewa sheep except Rump length and Tail length. Morphometric indices also revealed that West Shewa sheep are longer, heavier, and have a more

upright posture than North Shewa sheep, making them better suited for meat production.

The Myostatin and Callipyge genes were found to be polymorphic. The genotype frequencies variation and genetic diversity indices in both sheep populations showed that there will be an opportunity to select individual sheep with desired genotype and include it in the genetic improvement program. Besides, it will also help as baseline information for further investigation and to confirm the mutation of Callipyge and Myostatin genes in the studied populations. However, this is the first preliminary report in the North and West Shewa sheep populations. Hence, further studies with larger sample sizes and different ecotype of sheep populations are required to validate the current findings and explore the potential of these genes as genetic markers for meat production.

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Annex Table 1. Formula for calculating the morphometric indices in indigenous sheep populations.

Indices	Parameters used
Body index	Body length *100/chest girth
Dactyl thorax index	Canon Circumference/Chest Girth
Depth index	Chest depth/height at wither
Foreleg length	Wither height - chest depth.
Height index	Height at withers/body length
Height slope	Wither height - rump height
Body ratio	Height at withers/Height at rump
Length index	Body length/wither height
Over increase index	Height at rump/height at withers *100