ESAP Proceedings

LIVESTOCK SCIENCE IN DEVELOPMENT

Proceedings of the 27th Annual Conference of the Ethiopian Society of Animal Production (ESAP) Held at the Ethiopian Institute of Agricultural Research, Addis Ababa, August 29-31, 2019

Ethiopian Society of Animal Production (ESAP)
P.O.Box 62863, Addis Ababa, Ethiopia

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	Proceedings of the Workshop on the Establishment of the Pasture and Rangeland Forum Ethiopia (PaRFE)	2013

Livestock Science in Development

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Opening Speech

Honourable Guests,
ESAP Members and Participants, Ladies and Gentlemen,

On behalf of the Federal Democratic Republic of Ethiopia, Ministry Livestock and Fisheries and myself, I feel honoured in being amongst you today to officiate the opening of ESAP 27th Annual conference entitled “Livestock Science in Development”.

As it is well known to you all, the Federal Democratic of Ethiopia developed broader national development plans for all sectors that presumed to propel Ethiopia into middle income countries by 2025. As set forth in the second Growth and Transformation Plan (GTP II) and other strategies, reaching this goal will require boosting agricultural productivity, strengthening the industrial base and fostering export growth. Cognizant to this, the federal Democratic Republic of Ethiopia developed series of clear and broader national development plans that span over time horizons to 2025. Amongst these development plans is the Growth and Transformation Plans (GTP I and II), Climate Resilient Green Economy (CRGE), the Livestock Master Plan (LMP) and other strategic development goals that play key roles in supporting our ambitious national development goals.

Government realized that it is becoming increasingly important to improve the overall performance of the livestock development program to remain competitive in the international market. In this regards it is taking necessary policy and institutional measures to strengthen the administration of feed to increase the development of the feed industry and animal production and thereby enhance quality and safety of livestock products. In this regards, Veterinary Drug and Feed Administration and Control Authority and establishment of high Tech quality laboratories at federal level and various regulations and guidelines in place. This enhance the national capacity to regulate the proper production, distribution and use of veterinary drugs and feeds to ensure safety, and quality of the products and to enhance the productivity and health of the livestock population.

Ethiopian Society of Animal Production (ESAP) The 27th annual conference, this year took relevant theme, aims to create a forum for dialogue on Livestock Science in Development. It is expected that the national, as well as Global experiences on the topic will be shared at the plenary session. During the plenary session various invited papers from the government, private sectors and experienced professionals will be presented, to be followed by panel discussions.

Finally, I wish you all very pleasant and fruitful deliberations and discussion. I declare that the conference is officially opened.

Thank you!
Dr Gebre Egziabher Gebreyohanes,
State Minister, Livestock and Fisheries Resources Development, Ministry of Agriculture

Opportunities From the Genetic Diversity of the ILRI Genebank Forage Germplasm Collection

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Abstract:

The International Livestock Research Institute (ILRI) maintains a collection of 18,662 forage germplasmaccessions of grasses, herbaceous legumes and browse species at its Genebank in Addis Ababa, Ethiopia. Most of the collection was acquired from different regions, in partnership with and the consent of national genebanks, while some were donations from other institutes, notably the Commonwealth and Scientific Industrial Research Organization (CSIRO) in Australia. The focus of the forage germplasm activities in ILRI is on the conservation, characterization and use as animal feed of these resources in smallholder livestock systems. To this end, the determination of genetic diversity in the collection is essential, underpinning the development of trait-based subsets of accessions and for the identification of genotypes that can be used as parents to develop new germplasm containing specific traits of interest. Here we report on the use of genotyping-by-sequencing (GBS), a robust andaffordable genotyping method which uses a combination of genome complexity reduction using restriction endonucleases and next generation sequencing to identify large numbers of high-quality genome-wide genetic markers. GBS is a particularly useful technique to use on species with limited genomic information and we have applied this technique to assess genetic diversity in a range of our forage germplasm collections, including Napier grass (Cenchrus purpureus); Buffel grass (Cenchrus ciliaris); Rhodes grass (Chloris gayana); Lablab (Lablab purpureus); and Sesbania (Sesbania sesban). These data provide a significant resource for genetic and marker-trait association studies and genomicprediction, enhancing the prediction accuracy of superior genotypes and the efficiency of selection of new varieties, supporting improved animal production, using marker assisted breeding. Furthermore,the subsets are of a manageable size and can act as reference sets for distribution and evaluation in different agro-ecologies and production systems.

Key words: forage germplasm. genetic diversity. genotyping by sequencing. subset development and evaluation. trait phenotyping

Introduction

The International Livestock Research Institute (ILRI) maintains a collection of 18,662 forage germplasmaccessions of grasses, herbaceous legumes and browse species at its Genebank in Addis Ababa, Ethiopia. Most of the collection was acquired from different regions, in partnership with and the consent of national genebanks. Others were donations from other institutes, notably the Commonwealth and Scientific Industrial Research Organization (CSIRO) in Australia. The species rangefrom short-lived annuals to long-lived perennial plants that are adapted to the tropics and Mediterranean areas. ILRI maintains this collection on behalf of the international community, under the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA) framework (FAO, 2009).

A Global Tropical and Sub-Tropical Forages (TSTF) Strategy was developed in 2015 based on a

survey of major TSTF national and international genebanks and the input from a workshop of genebank managers and forage specialists (Pengelly and Maass, 2017). The strategy attempted to balance the higher-level objectives of improving both the conservation and use of forage germplasm for forage or environmental use via three themes: (1) Building a global community of genebanks and forage utilisation researchers; (2) Achieving greater efficiency and security in genebank operations to ensure conservation of the most important TSTF genera and species and better understanding of their diversity; and (3) Attaining greater utilisation of this valuable collection in the developed and developing world. Amongst the recommendations for the implementation of this strategy was that the genebanks should adopt a species prioritisation (table 1) and apply this prioritisation to accession management, including the appropriate removal of duplicates and the archiving or equivalent of low priority species, so that limited resources can be better applied to taxa most likely to contribute to impacts in improved livestock production and resource management (Pengelly and Maass, 2017). The number of ILRI accessions in each of the categories is shown in table 2.

Table 1. Prioritization categories and their definition

Category	Definition/explanation of species' category
1	Species of known high value, included in the Tropical forages database (www.tropicalforages.info) or commercially useful somewhere
2	Identified as high potential for further development towards commercial use or emerging as one of high value somewhere
3	Often thought of as being interesting, but never with enough value to advance to category 1or 2
4	Recognized anywhere as being of importance through its taxonomic affinity to (even minor) crop species (crop wild relatives, CWR)
5	Widely recognized as being of low value for forage or environmental use

Table 2: Distribution of ILRI accessions in the Tropical and Sub-Tropical Forages Strategy prioritization catagories

Prioritisation Category	Number of accessions
1	6132
2	551
3	2003
4	771
1, 2, 3	13
1, 4	724
2, 4	195
3, 4	170
3, 5	94
4, 5	3
5	4203
Temperate /Mediterranean	1182
Trees	370
Others	2229
Total	**18640**

Most of the forage accessions in the ILRI Genebank collections have rarely or never been requested and distributed, and this is possibly so, at least in part, because most of these accessions have not been effectively characterised to better understand their forage value. This could be the reason for moving some of those uncharacterised accessions into the low prioritisation categories, for example, the more than 4000 accessions in category 5 (table 2). The focus of the forage germplasm activities in ILRI is on the conservation, characterization and use as animal feed of these resources in smallholderlivestock systems. The determination of genetic diversity in the collection is essential for the development of trait-based subsets of accessions, and for the identification of genotypes that can beused as parents to develop new germplasm containing specific traits of interest. Here we report on the use of genotyping-by-sequencing (GBS), a robust and affordable genotyping method which uses acombination of genome complexity reduction with restriction endonucleases, enzymes that cut the DNA at specific sites, and next generation sequencing to identify large numbers of high-quality genome-wide genetic markers that are suitable for diversity analysis, marker-trait associations and genomic prediction.

Materials and Methods:

Genotypic analysis

Grass and legume species with good or potential forage value were selected and subjected to GBS using the DArTseq platform (Kilian et al., 2012). Three grass and two legume species from the ILRI forage Genebank have been included in the study to date. The grasses included: 105 accessions of Napier grass (*Cenchrus purpureus*); 185 accessions of Buffel grass (*Cenchrus ciliaris*) and 104 accessions of Rhodes grass (*Chloris gayana*). The Napier grass collection consisted of 60 accessions from ILRI and 45 accessions from EMBRAPA, Brazil. The legumes included: 145 accessions of Hyacinthbean (*Lablab purpureus*) and 171 accessions of Egyptian pea (*Sesbania sesban*).

Genotypic data were generated through the application of GBS on the DArTseq platform that combines genome complexity reduction using a combination of restriction enzymes and next-generation sequencing (Kilian et al., 2012). DNA was isolated from one plant per accession of the grass species and 15 plants per accession, to assess the level of diversity contained both within and between accessions, of the legumes. The genomic DNA (approximately 50 ng) was digested with a combinationof *PstI/HpaII* restriction endonucleases and the resulting fragments were ligated to a *PstI* overhang compatible oligonucleotide adapter and sequenced on an Illumina HiSeq 2500 platform (Illumina Inc.) using *PstI* site-specific primers. Short sequence fragments, SilicoDArT (presence/absence) and SNP markers were generated following the DArTseq protocol. Data were analysed using R tools and otherstatistical analysis software to identify diversity, population structure and subsets, as described by Muktar et. al., (2019).

Phenotypic analysis

Field phenotyping of the Napier grass collection for agronomic traits is as described by Habte et al., 2019 (*in preparation*). Napier grass collections consisting of 84 (59 ILRI and 25 EMBRAPA) accessions were planted at the ILRI field Genebank in Bishoftu, Ethiopia using a partial replication design with fourreplications. After establishment at the beginning of the main rains and an initial harvest six months later in the dry season of 2018, drought stress was imposed on the established field plants in such a way that two replicates were watered using a drip irrigation system to replace the loss of water due to evapotranspiration (volumetric soil moisture content of 20 %), i.e., optimal

water (OW) and the other two replicates were irrigated with a limited amount of water (volumetric soil moisture contentof 10 %), i.e., water deficit (WD). The soil moisture content of both water regimes was monitored usinga Delta soil moisture probe (HD, England). The physiological drought stress effect was also monitored using a portable chlorophyll fluorescence meter Handy PEA (Hansatech, UK) to analyze the photochemical efficiency of leaves growing under stress. The trial was harvested and data on morphological traits, agronomic performance and feed quality were collected following every 8 weeksof regrowth.

Subset identification

To select a subset of representative accessions of Napier grass, the R package Core Hunter v. 3.2.1 (De et al., 2018) was used. This program identifies core subsets using diverse allocation strategies by optimizing many genetic parameters simultaneously. The modified Roger's distance (RD), Shannon's information index (SH), average entry-to-nearest-entry distance (EN), expected proportion of heterozygous loci (He) and allele coverage (CV), each with an equal weight, were used to define a coresubset representing the entire collection. Field phenotyping of the other species is planned following results of genotypic analysis.

Results

GBS analysis and molecular marker development

Results are as described by Muktar et. al., (2019) and Negawo et al., (2018). Both SNP and SilicoDArT genome-wide markers were generated for the different forage species (table 3). The short sequences of the generated markers were aligned with reference genomes of closely related species (table 3). In Napier grass, a total of 85,452 SNP and 116,190 SilicoDArT genome-wide markers were called on the 105 accessions with an average call rate of 87 % for SNPs and 95 % for SilicoDArT markers. Missing values ranged from 0 to 59 % for SNPs, and from 0 to 30 % for the SilicoDArT markers, with an averagevalue of 15 % in both marker sets. Accession ILRI_16621 had the highest missing value content (74 %)and was excluded from further analysis. Approximately 42 % (48,536) of the SilicoDArT and 20 % (17,086) of the SNP markers had a polymorphic information content (PIC) value above 0.25. The short sequence reads, averaging 55 nt in length, corresponding to each marker were mapped on to the pearlmillet reference genome and genomic position information was generated for 17 % of the SNP and 33 % of the SilicoDArT markers.

The genotypic data for Buffel grass and Rhodes grasses were analysed as described for Napier grass above. For Buffel grass, 111,917 SilicoDArT and 93,501 SNP markers were obtained for 185 accessions. Out of those markers, 8,053 (7 %) SilicoDArT and 15,465 (16 %) SNP markers were aligned with *Setariaitalica* as a reference genome. For Rhodes grass, a preliminary analysis has generated 93,128 SilicoDArT and 65,529 SNP markers from 94 accessions. Of the three selected reference genomes, more markers (0.74 % SilicoDArT and 5.86 % SNP markers) were able to be aligned on the teff (*Eragrostis tef*) reference genome followed by Manila grass (*Zoysia matrella*) (0.56 % Silico DArT and 5.13 % SNP markers). The least number of markers (0.23 % Silico DArT and 2.07 % SNP markers) were mapped to the *Setaria italica* reference genome. For *Sesbania sesban* 34,798 SilicoDArT and 47,609 SNP markers were generated. Relatively few markers (1,168 SilicoDArT and 2,460 SNP markers) were aligned with the *Glycine max* reference genome.

Table 3. Markers generated GBS studies in forage crops and percentage of the mapped markers onto the selected reference genomes.

Species	Number of accessions genotyped	Number of markers		Number of Mapped markers (%)		Reference genome
		Silico DArT	SNP	Silico DArT (%)	SNP (%)	
Cenchrus purpureus	105	116,190	85,452	17	33	*Penissetum glaucum* (pearl millet)
Cenchrus ciliaris	185	111,917	93,501	7.2	16.3	*Setaria italica* (foxtail millet)
Chloris gayana	94	93,128	65,529	0.23	2.07	*Setaria italica* (foxtail millet)
				0.74	5.86	*Eragrostis tef* (teff)
				0.56	5.13	*Zoysia matrella* (Manila grass)
Sesbania sesban	41	34,798	47,609	3.36	5.17	*Glycine max* (Soybean)

For *Lablab purpureus* a total of 1,843 samples generated from 142 accessions (1 to 29 plants per accession) were genotyped. The genotyping produced a total of 38,824 SNP and 64,793 silicoDArT genome-wide and high-density markers. Out of the 142 accessions, 108 were represented by 10 or more plants per accession, and 72 were represented by 15 or more plants per accession. These will be used for the study of within and between accession diversity, an analysis which is currently being undertaken. Distribution of PIC and He values of the markers are shown in figure 5.

Diversity and population structure

In the Napier grass population, diversity and population structure have previously been evaluated and presented using 980 highly polymorphic and independent SNP markers (pruned for LD at $r^2 = 0.5$) distributed across the genome, which were selected from the 85,452 genome-wide SNP markers (Muktar et al., 2019). The presence of subpopulations within the accessions was analysed with the 980 SNP markers described above, using the software STRUCTURE, PCA and UPGMA clustering methods. The analysis revealed the presence of between 2 and 7 sub-groups in the Napier grass population. The STRUCTURE analysis detected two major groups, with the collection from ILRI predominantly represented in Group I and most of the EMBRAPA collections assigned to Group II. However, this analysis also indicated the presence of up to 5 possible sub-groups, described in detail in Muktar et al., 2019. UPGMA further clustered the accessions into seven sub-groups (figure 1a), and Groups I, II, III, V, and VI were highly consistent with the STRUCTURE classification (figure 1b). Group IV and VI mainly consist of materials from ILRI and Groups I, II and III are mainly EMBRAPA materials, with Groups V and VII containing material from both collections. The eight C. *purpureus* x *P. glaucum* hybrids were distributed across groups IV (ILRI_16835 and ILRI_16837), V (ILRI_16834 and ILRI_16838), and VI (ILRI_15357, ILRI_16840, ILRI_18662 and ILRI_14982).

For Buffel grass, diversity and population structure analysis using 1,000 selected SNP markers distributed across the reference genome revealed the presence of two main groups with further sub-groups in the collection (figure 2 a and b). For Rhodes grass, cluster analysis using 10,111 SNP markers with no missing data clearly showed twodifferentiated groups (figure 3). A preliminary cluster analysis of the *Sesbania sesban* data with SNP markers filtered for polymorphic information content (PIC) ≥0.2 and missing value ≤30% is shown in figure 4.

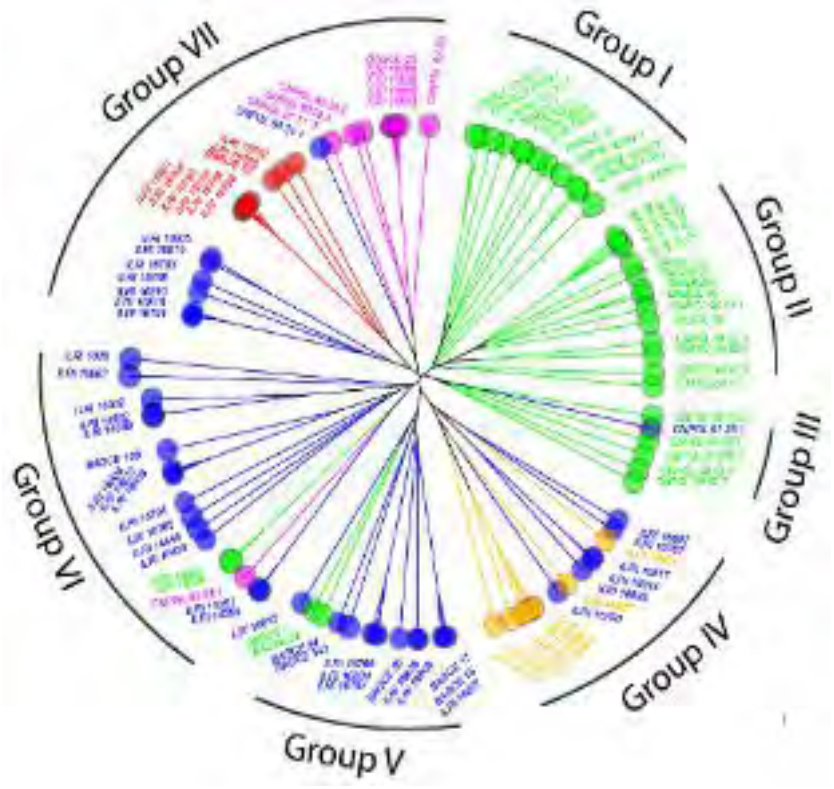
Phenotyping morphological traits:

The performance of Napier grass genotypes for agro-morphological and feed quality traits were assessed over three wet and three dry seasons harvests during 2018. Significant variations were observed among the genotypes for plant height, leaf size, stem diameter, tiller number, biomass yield and water use efficiency, that indicated the existence of phenotypic variability among the experimental accessions (figures 6). Similarly, results from forage quality analysis from leaf and stem tissues showed significant differences among genotypes, particularly for Acid detergent fibre (ADF), Neutral detergent fibre (NDF), Acid detergent lignin (ADL), Organic matter (OM), Dry matter (DM), Total nitrogen (N), Crude protein (CP), *In vitro* organic matter digestibility (IVOMD) and Metabolizable energy (Me). These results indicate a substantial opportunity for the improvement of different forage quality traits in Napier grass (figure 7).

Identification of sub-sets

Mini core subsets of Napier grass were identified using a combined analysis of genotypic and phenotypic data based on 68 accessions (Muktar et al., 2019). The initial phenotypic trait data (table 4) were used to complement the selected 980 genome-wide SNP marker data in the analysis. UPGMA analysis clustered the 68 accessions into seven sub-groups, and each sub-group was well represented in the subsets. Forage biomass traits, total fresh weight per plant (TFWPP) and total dry weight per plant (TDWPP), were highly variable among accessions in the sub-groups. Groups II and IV had highermean values while groups I and VII had lower mean values for both traits when grown under optimal- water conditions. A similar trend was observed when grown under water-deficit conditions, except that group IV had an average mean value in this case.

a



b

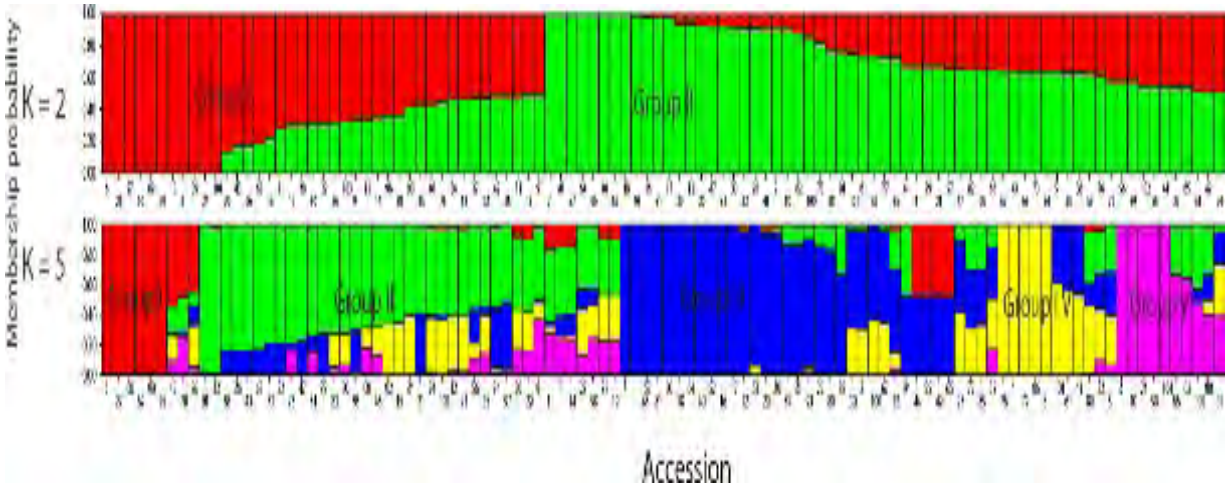


Figure 1. Clusters of 104 Napier grass accessions using selected SNP markers: (a) UPGMA tree showing seven groups; (b) Bar plots based on the admixture model in STRUCTURE, for K = 2 and K = 5. Colours in (a) are according to the STRUCTURE analysis with k = 5. (Muktar et al., 2019).

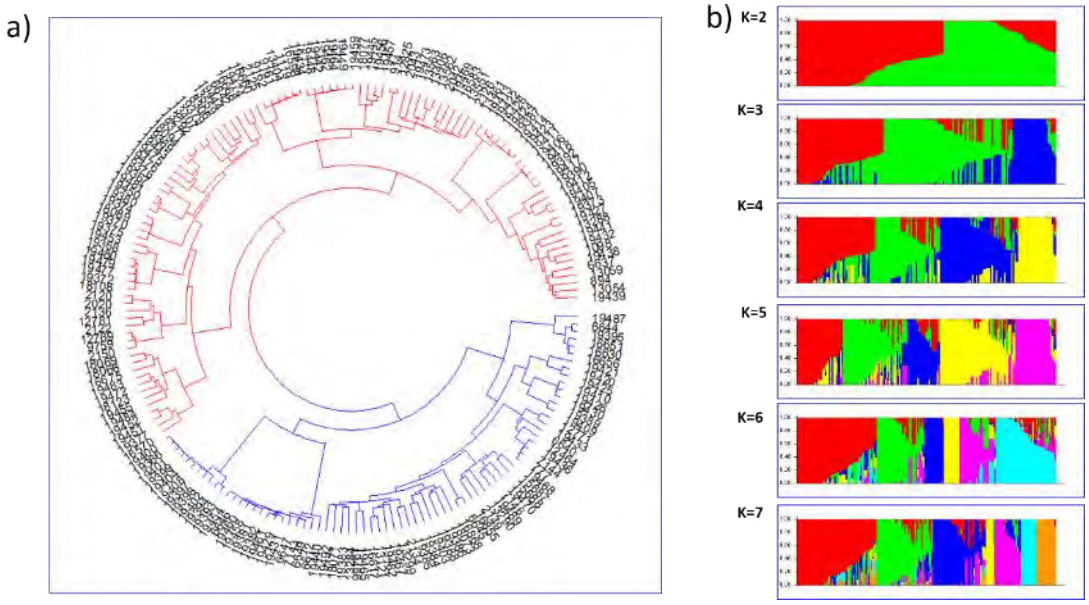


Figure 2. Population structure of Buffel grass using 1,000 selected SNP markers with (a) cluster analysis of the 185 accessions and; (b) bar plots showing the suggested subpopulations.

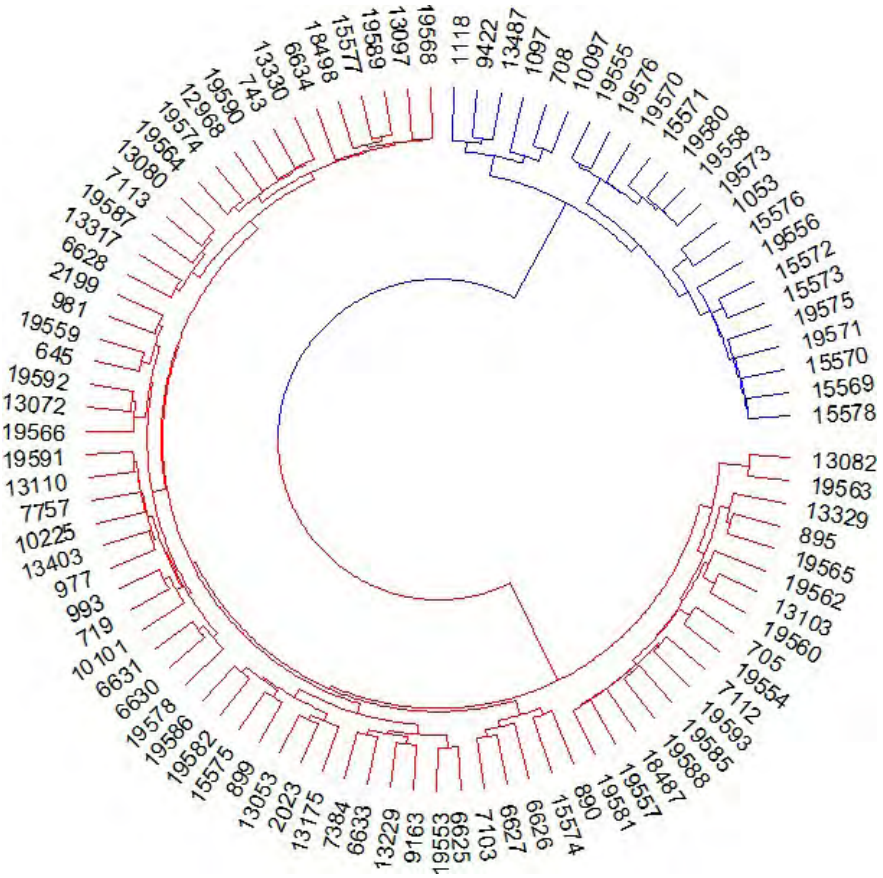


Figure 3. Cluster analysis of the 94 Rhodes grass accessions using SNP markers clearly showing two differentiated groups.

Table 4. Mean phenotypic data of accessions used for subsetting the Napier grass collection (Muktaret al., 2019)

Genotype	Fv/Fm		PI		TFWPP(g/plant)		TDWPP(g/plant)	
	OW	WD	OW	WD	OW	WD	OW	WD
ILRI_1026	0.72	0.61	1.7	1.25	122.9	58.76	34.62	19.81
ILRI_14355	0.74	0.69	2.85	2.11	310.67	224.67	94.06	62.24
ILRI_14389	0.75	0.72	3.06	1.77	194.93	104.16	56.24	29.25
ILRI_14982	0.74	0.68	2.99	1.94	307.7	149.82	79.19	41.41
ILRI_14983	0.73	0.69	3.41	1.97	304.76	190.68	72.94	50.11
ILRI_14984	0.71	0.7	2.58	2.16	387.9	189.24	111.13	57.27
ILRI_15357	0.75	0.73	3.55	3.25	294.03	167.24	81.51	48.42
ILRI_15743	0.75	0.7	3.38	2.26	221.7	167.34	55.6	44.5
ILRI_16782	0.74	0.76	3.25	4.7	187.87	139.94	51.21	35.1
ILRI_16783	0.56	0.67	1.09	1.78	284.47	72.89	77.67	19.8
ILRI_16784	0.74	0.69	3.01	2.06	184.81	181.25	49.64	47.83
ILRI_16785	0.7	0.69	1.64	1.37	315.95	194.94	84.61	63.68
ILRI_16786	0.71	0.72	1.69	2.78	322.39	203.85	94.42	61.98
ILRI_16787	0.74	0.68	2.14	1.38	262.44	83.55	72.11	23.94
ILRI_16788	0.69	0.69	1.27	1.61	142.69	145.63	39.17	44.41
ILRI_16789	0.71	0.68	2.18	2.44	342.49	201.72	97.59	59.6
ILRI_16790	0.72	0.66	2.84	2.37	76.02	37.01	17.99	9.97
ILRI_16791	0.75	0.7	3.77	2.71	291.99	313.16	79.01	87.85
ILRI_16792	0.73	0.68	2.35	2.38	347.47	291.4	102.81	83.71
ILRI_16793	0.73	0.73	3.25	3.71	375.99	181.42	111.22	50.12
ILRI_16794	0.77	0.73	5.37	4.82	264.5	161.28	75.71	48.91
ILRI_16795	0.74	0.71	2.79	3.02	322.89	203.99	93.51	60.72
ILRI_16796	0.76	0.71	4.71	2.43	135.45	65.7	63.1	19.53
ILRI_16797	0.7	0.69	2.36	2.26	13.78	47.71	3.29	12.06
ILRI_16798	0.73	0.71	2.58	2.53	325.68	236.78	88.49	69.74
ILRI_16799	0.72	0.7	1.91	1.75	120.12	64.36	29.91	17.15
ILRI_16800	0.75	0.7	2.9	2.11	413.65	251.75	127.19	74.77
ILRI_16801	0.72	0.72	1.72	2.71	434.76	202.42	126.66	58.48
ILRI_16802	0.74	0.71	3.6	2.85	276.79	287.27	75.02	77.49
ILRI_16803	0.7	0.7	1.69	1.83	380.05	200.15	111.7	62.73
ILRI_16804	0.73	0.71	2.43	1.71	391.56	82.84	101.7	22.05

Genotype	Fv/Fm		PI		TFWPP(g/plant)		TDWPP(g/plant)	
	OW	**WD**	**OW**	**WD**	**OW**	**WD**	**OW**	**WD**
ILRI_16805	0.73	0.72	2.49	2.33	39.55	46.94	8.36	13.58
ILRI_16806	0.73	0.67	2.93	2.32	416.31	155.13	117.92	51.68
ILRI_16807	0.7	0.69	1.96	2.49	183.95	137.32	47.14	35.78
ILRI_16808	0.75	0.71	3.87	3.11	104.16	65.47	31.6	19.18
ILRI_16809	0.73	0.64	2.93	2.29	161	107.06	47.19	32.36
ILRI_16810	0.69	0.72	2.11	2.25	185	128.67	40.28	38.37
ILRI_16811	0.74	0.68	2.91	1.75	291.16	108.54	78.34	30.91
ILRI_16812	0.69	0.73	2.06	4.15	190.4	86	52.28	21.98
ILRI_16813	0.74	0.64	5.25	1.5	137.93	153.37	26.53	40.48
ILRI_16814	0.7	0.71	2.39	3.57	291.55	139.95	73.11	36.63
ILRI_16815	0.75	0.64	3.9	1.23	239.01	100.2	65.49	27.11
ILRI_16816	0.76	0.76	3.75	4.94	118.12	62.08	34.35	17.49
ILRI_16817	0.75	0.69	3.56	1.72	198.56	82.35	51.45	23.84
ILRI_16818	0.75	0.71	3.72	2.5	109.2	96.1	31.71	29.81
ILRI_16819	0.75	0.71	5.02	1.88	366.63	266.45	104.47	73.15
ILRI_16821	0.72	0.72	2.5	2.97	123.08	106.05	33.53	31.03
ILRI_16822	0.72	0.74	2.28	3.24	96.01	103.92	26.19	31.8
ILRI_16834	0.75	0.73	3.1	2.47	194.79	53.76	54.93	15.97
ILRI_16835	0.71	0.69	1.92	2.43	137.03	44.21	33.24	12.76
ILRI_16836	0.76	0.69	2.63	1.84	306.78	150.31	71.3	47.23
ILRI_16837	0.73	0.66	2.55	1.11	225.16	164.55	60.73	41.64
ILRI_16838	0.75	0.75	3.89	3.74	131.98	111.62	33.96	31.83
ILRI_16839	0.7	0.7	2.22	2.56	411.17	128.22	94.35	35.18
ILRI_16840	0.71	0.68	1.89	1.11	241.16	104.71	59.81	29.37
ILRI_16902	0.77	0.72	3.97	3.23	207.22	173.02	61.07	50.36
ILRI_18438	0.7	0.73	2.79	3.28	248.14	214.48	70.41	57.58
ILRI_18448	0.7	0.72	3.65	3.62	141.79	76.48	42.49	23.24
ILRI_18662	0.76	0.63	3.96	1.89	4.55	38.94	1.7	7.92
BAGCE_100	0.67	0.73	2.25	2.77	240.39	179.33	65.59	61.91
BAGCE_17	0.71	0.59	2.07	1.34	144.31	108.33	38.16	27.61
BAGCE_30	0.69	0.75	1.92	3.57	368.22	254.29	89.49	70.84
BAGCE_343	0.76	0.77	3.36	5.03	241.19	111.35	65.92	31.58
BAGCE_53	0.74	0.72	2.73	2.54	344.48	91.93	83.73	24.93
BAGCE_81	0.74	0.68	2.73	1.56	229.01	62.61	56.36	17.59
BAGCE_86	0.73	0.71	3.32	2.34	274.05	123.33	69.15	32.93
BAGCE_90	0.72	0.71	2.69	2.36	385.86	133.88	93.74	35.84
BAGCE_97	0.74	0.7	3.06	2.32	191.56	126.51	45.31	34.67
Maximum	**0.77**	**0.77**	**5.37**	**5.03**	**434.76**	**313.16**	**127.19**	**87.85**
Minimum	**0.56**	**0.59**	**1.09**	**1.11**	**4.55**	**37.01**	**1.7**	**7.92**
Average	**0.73**	**0.70**	**2.86**	**2.49**	**239.40**	**139.71**	**65.01**	**39.86**

Fv/Fm =quantum efficiency of photosystem II, PI = performance index, TDWPP=total dry weight per plot,TFWPP= total fresh weight per plot.

A subset of 14 (20%) accessions representing the range of phenotypic and genetic diversity in the 68 Napier grass accessions was identified for both optimal-water and water-deficit conditions and seven accessions are common between the two subsets (table 5). Mini core subsets of Buffel grass were also created based on the genotypic (silicoDArT and SNP) data generated from the collection and some historical feed quality data (not shown). The 'corehunter' R package was used for the purpose. Subset analysis for the other species will be undertaken following completion of the full genetic diversity analysis and evaluating the accessions for agronomic performance and nutritional qualities in the field.

Table 5. Napier grass subsets representing the diversity in the collection from the ILRI Genebank for evaluation under irrigated and water deficit conditions (Muktar et al., 2019)

Optimal water			Water-deficit		
NAME	**Species**	**Origin**	**NAME**	**Species**	**Origin**
ILRI_1026*	*C. purpureus*	Burundi	ILRI_1026*	*C. purpureus*	Burundi
ILRI_16840*	*C. purpureus x P. glaucum*	Zimbabwe	ILRI_14389	*C. purpureus*	Nigeria
ILRI_14982	*C. purpureus x P. glaucum*	USA	ILRI_14983	*C. purpureus*	USA
ILRI_14984	*C. purpureus*	USA	ILRI_16811	*C. purpureus*	USA
ILRI_16793*	*C. purpureus*	Cuba	ILRI_16791	*C. purpureus*	Swaziland
ILRI_16794	*C. purpureus*	Mozambique	ILRI_16793*	*C. purpureus*	Cuba
ILRI_16814*	*C. purpureus*	USA	ILRI_16816	*C. purpureus*	USA
ILRI_16839	*C. purpureus*	Zimbabwe	ILRI_16796	*C. purpureus*	Zimbabwe
ILRI_16819	*C. purpureus*	USA	ILRI_16806*	*C. purpureus*	USA
ILRI_16797	*C. purpureus*	Zimbabwe	ILRI_16782	*C. purpureus*	Tanzania
ILRI_16806*	*C. purpureus*	USA	ILRI_16814*	*C. purpureus*	USA
ILRI_16822	*C. purpureus*	Malawi	ILRI_16840*	*C. purpureus x P. glaucum*	Zimbabwe
BAGCE_30*	*C. purpureus*	Brazil	BAGCE_30*	*C. purpureus*	Brazil
BAGCE_97*	*C. purpureus*	Brazil	BAGCE_97*	*C. purpureus*	Brazil

*All the accessions are collections of ILRI, except BAGCE_37 and BAGCE_97 which are collected by EMBRAPA, *accession selected in both subsets*

Discussion:

ILRI maintains a collection of more than 18,600 forage germplasm accessions of grasses, herbaceous legumes and browse species at its Genebank in Addis Ababa, Ethiopia, as a public good for use for agriculture, research and education for food security. Understanding of genetic diversity in this collection is essential for its management and utilisation in plant breeding, other research and direct use for feed production and security. The determination of genetic diversity in the collection is also essential for the development of trait-based subsets of accessions, and for the identification of genotypes that can be used as parents to develop new germplasm containing specific traits of interest. Furthermore, identifying heterozygosity within selected collections of accessions held in trust will

generate information that will facilitate the establishment of a baseline for the diversity of the collections across multiple crops, will be useful for exploring crop evolution, and will support forage plant breeding and genebank management. Genotyping is perceived as a tool to comprehensively characterize collections and reveal the diversity and population structure within and among germplasm accessions. Here we report on the use of genotyping-by-sequencing (GBS), a robust and affordable genotyping method which uses a combination of genome complexity reduction with restriction enzymes and next generation sequencing to identify large numbers of high-quality genome-wide genetic markers that are suitable for diversity analysis, marker-trait associations and genomic prediction. GBS is a particularly useful technique to use on species with limited genomic information and we have applied this technique to assess genetic diversity in a range of our forage germplasm collections, including Napier grass; Buffel grass; Rhodes grass; Lablab; and Sesbania. The above species are amongst the germplasm that are most frequently requested and distributed from the ILRI Genebank, and they are all in category 1 of the Global Tropical and Sub-Tropical Forages (TSTF)Strategy species prioritisation list (Tables 1 and 2).

GBS analysis revealed a significant amount of diversity held in a small collection of Napier grass accessions (Muktar et al., 2019) and also in the Buffel grass collection (Negawo et al., 2019). The datafor Rhodes grass, Sesbania and Lablab are currently being analysed, and a preliminary analysis has indicated the presence of diversity in the collections of Rhodes grass (figure 3) and Sesbania (figure 4).

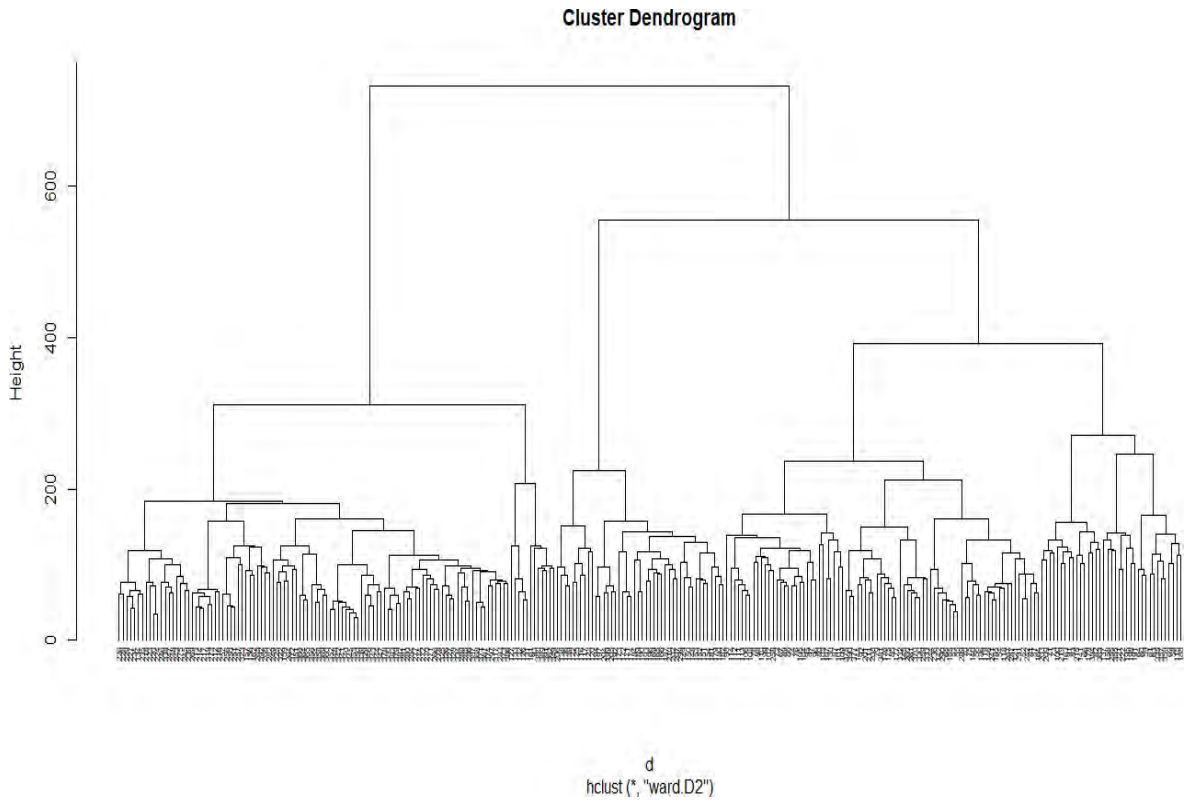


Figure 4. Preliminary cluster analysis of the Sesbania sesban collection using SNP markers.

In Napier grass, genetic diversity and population structure analyses revealed the existence of a substantial amount of variation in the collection (figure 1). This supports previous work by Negawo et al., (2018) who previously identified genetic diversity in this collection using microsatellite markers. The presence of two to seven groups was observed by STRUCTURE, principal component (PCA) and phylogenetic analyses and most of the materials from ILRI and EMBRAPA grouped separately. Analysisof molecular variance (AMOVA) indicated that the seven groups detected are significantly different from each other, with up to 14 % variation among the groups. The high level of diversity and population stratification observed could be attributed to the outcrossing, self-incompatibility (Hannaet al., 2004) and polyploid nature of Napier grass. Furthermore, selection, breeding systems, and variation in geographical origin may also be contributing to the variation seen between the materialsderived from the ILRI and EMBRAPA collections.

Phenotypic analysis of morphological traits, agronomic performance and feed quality characteristics in Napier grass indicated the existence of phenotypic variability among the experimental accessions that would potentially identify highly productive accessions for promotion in support of livestock production both in water stressed and irrigated forage production areas. This adds to a previous study by Wouw et al. (1999) that also identified phenotypic variation in the ILRI collection.

In the Buffel grass collection, diversity and population structure are shown in figure 2. Two main groups were identified, with the possibility of identifying additional sub-groups, in the collection. Here too a previous study by Ricardo et al. (2017) had identified phenotypic variation in this collection andefforts are underway to put these various sources of data together for a more comprehensive analysisof the collection.

Sub-setting

In Napier grass, subsets were identified based on a combined analysis of GBS and phenotypic data. Only a few accessions were selected for the subsets but they well represent the overall genetic and phenotypic diversity of the collections. Based on a stress tolerance index (Fernandez, 1992) and water use efficiency analysis in the water deficit experiment in the 2018 dry season, accessions 16791, 16792, 16800, BAGCE100, 16801, 16802, 16819, 18438, 16786 and CNPGL 93-37-5 showed higher WUE (figures 6) and as such offer potential candidates for improved performance under dry conditions.

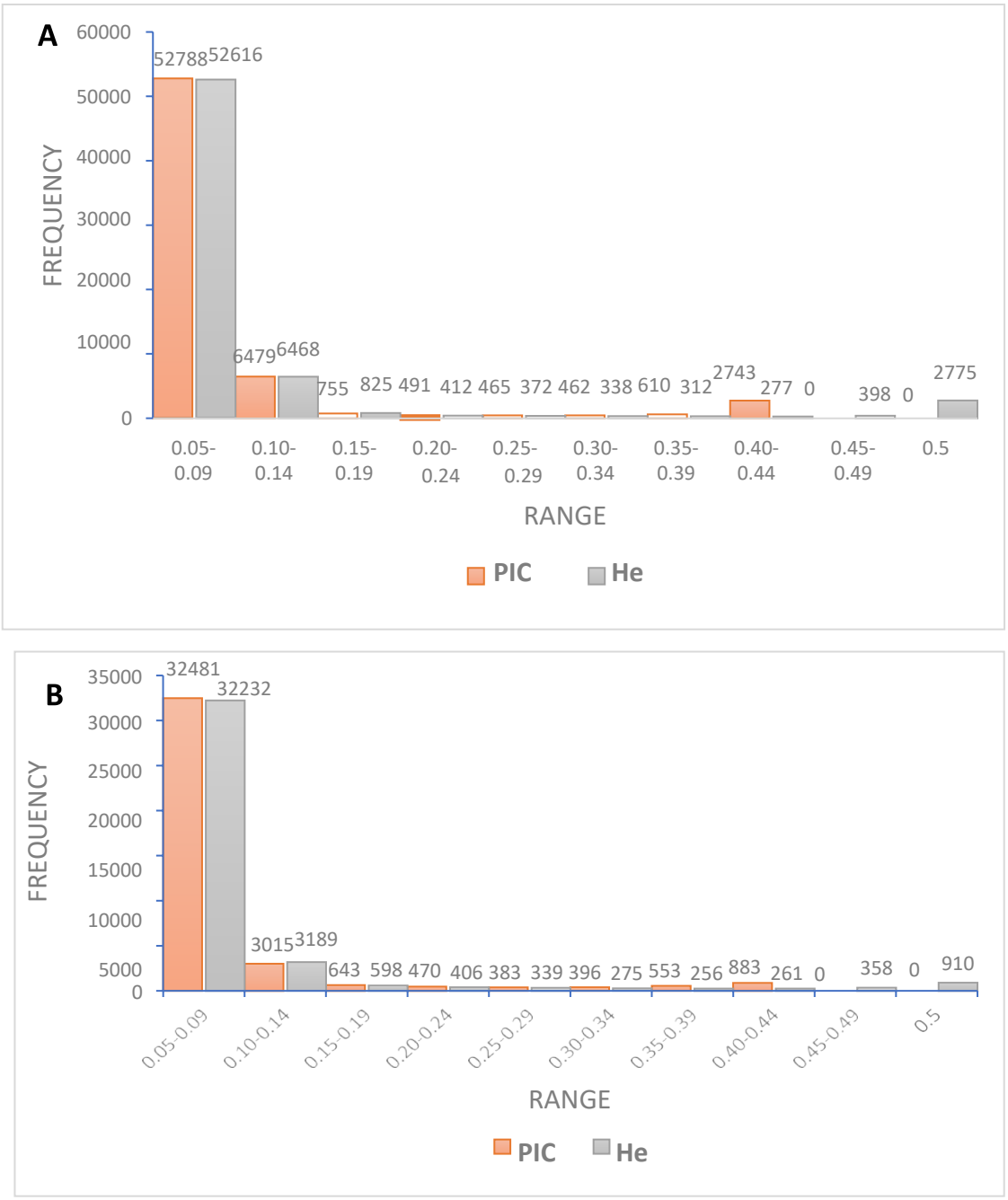


Figure 5. Distribution of polymorphic information content (PIC) and heterozygosity (He) for the silicoDArT (A) and SNP (B) markers in Lablab purpureus

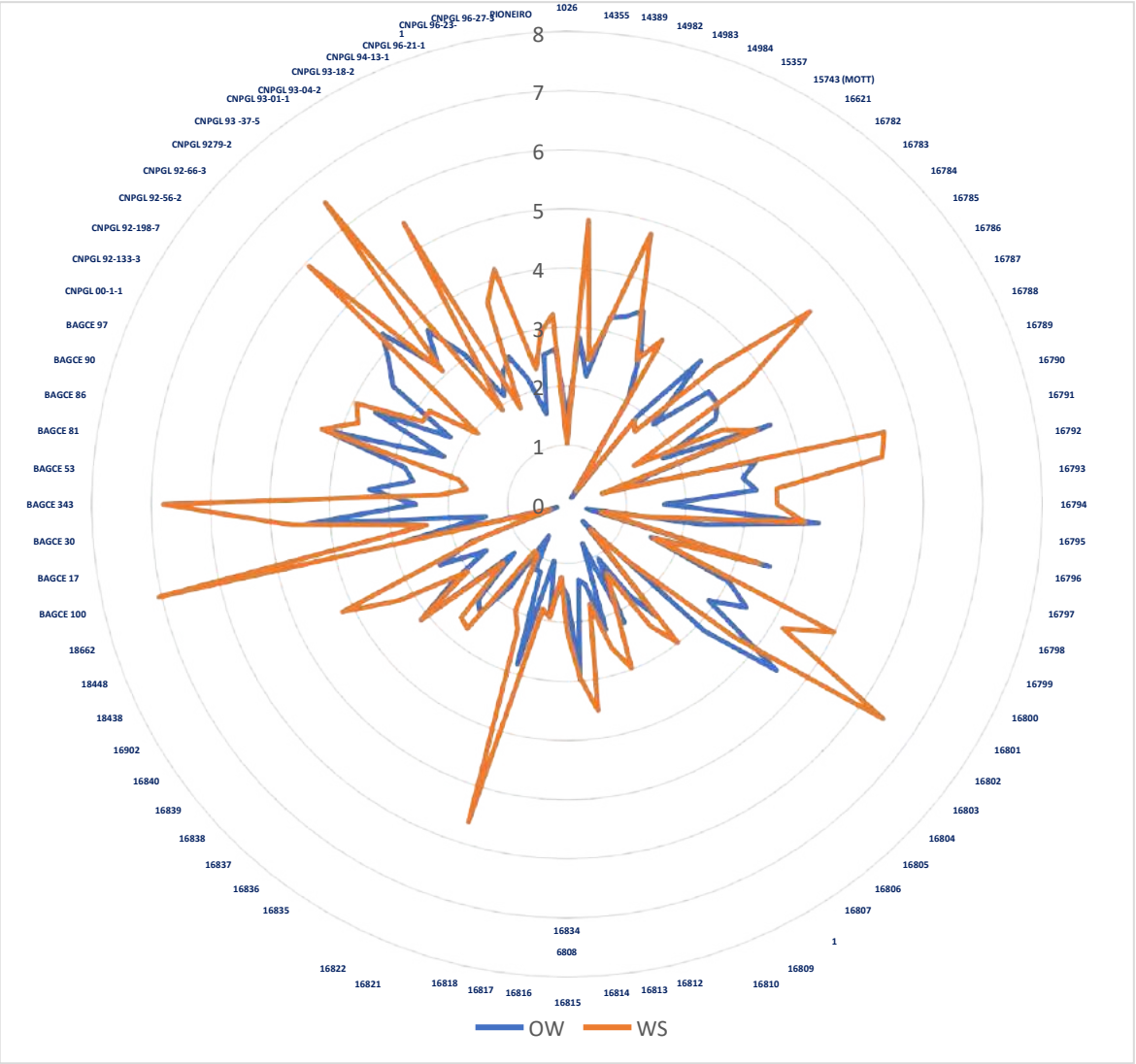


Figure 6. Water use efficiency (g/m3) of Napier grass genotypes grown under optimum water (OW) and water stress (WS) conditions across dry season harvests.

For high biomass production during irrigation accessions 16791, 16819, 16802, 14983, 16814, 16783, BAGCE 100, BAGCE 30, CNPGL 00-1-1 and CNPGL 92-198-7 were identified as potential promising lines. The high biomass producing genotypes were either tall plants or they produced many tillers, indicating that high biomass production is associated with plant height and tiller number in Napier grass. These accessions offer prime candidates for further evaluation in different areas and production systems. Evaluating these subsets, which consist of only a few genotypes, will save time and resources when compared to evaluating the whole collection for target traits. The subsets can also serve as reference sets, representing the genetic diversity of the whole collection. Trait specific subset identification for the other species will follow the analysis of GBS data, field phenotyping and feed quality analysis and the combined analysis of genotypic and phenotypic data.

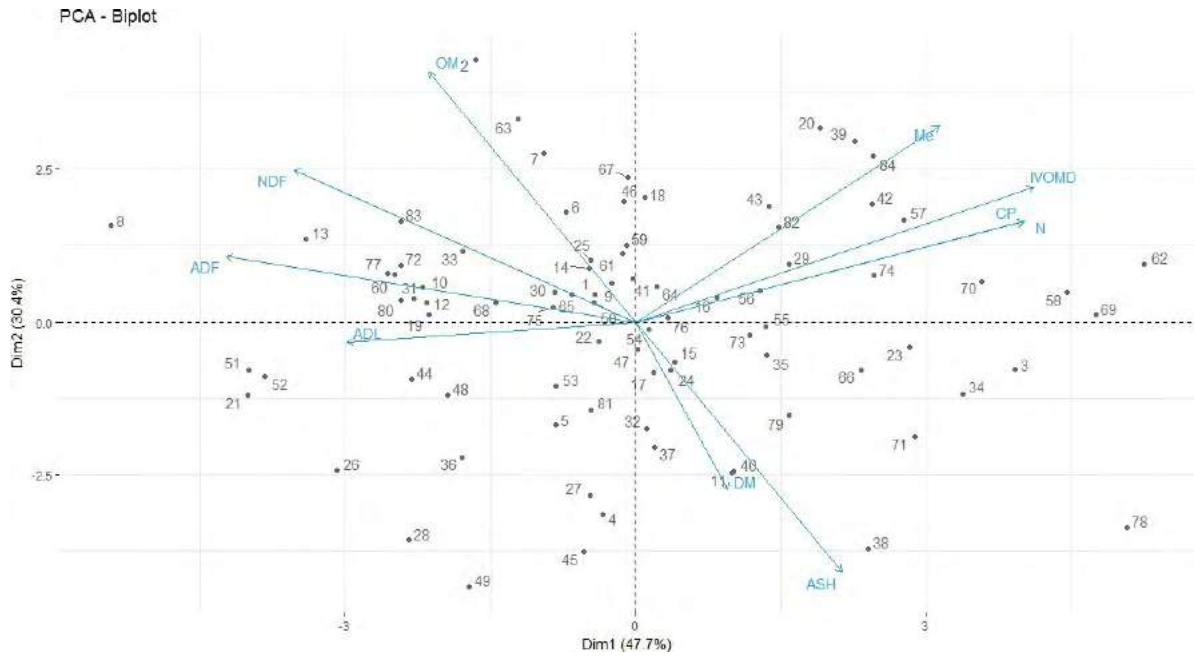


Figure 7. PCA biplot showing feed quality traits of Napier grass accessions: Acid detergent fibre (ADF); Neutral detergent fibre (NDF); Acid detergent lignin (ADL); Metabolizable energy (Me); Organic matter (OM); Dry matter (DM); Total nitrogen Crude protein (CP); In vitro organic matter digestibility (IVOMD).

Conclusion:

We have used GBS to identify the diversity held in forage germplasm collections of Napier grass and Buffel grass. Phenotypic assessment of the Napier grass collection on the field for agronomic traits enabled the identification of subsets of accessions for drought tolerance and biomass productivity. Evaluation of these subsets across ecologies and seasons may further lead to the identification of best-bet accessions for those environments. A large number of SNP and SilicoDArT markers were generated that could be useful for diversity assessment in germplasm collections of other collections and related forage species.

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Innovative Feed Solutions the Ethio-Feed Plc Ten Years' Experience: - "The Quest for Research Agenda"

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Abstract

Ethio-Feed PLC is a private livestock feed manufacturing company established by professionals engaged in the livestock industry. The driving factors for establishing the company include i) contribute to production and productivity of milk, meat and eggs, ii) ensure survival of the core breeding stock in the pastoral and agro-pastoral areas, iii) improve health and performance of the draft oxen and equines in particular horses. Currently, the company produces and market different kinds of livestock feeds for dairy, fattening, poultry, equines as well as drought supplementary feed. As one of the private commercial feed company, what makes the company preferable is its use of "Innovate feed solution" for the production and marketing of different types of livestock feed products.

Introduction

Feed cost accounts for 60 to 70% of the total cost of livestock production. As a result, shortage of feed and escalating price of feeds is adversely affecting the productivity and profitability of commercial livestock operations. The situation has a far-reaching effect on the business and profitability of feed industries as their operations would become irregular with frequent disruption by shortage and escalating price of feed ingredients, which would raise their cost of production beyond what the livestock producers can afford to buy (Adugna et al, 2013).

The Eight *holistic and integrated* "*Innovate feed solution*" approach that make the company preferable include: 1) Production of quality and affordable total mixed ration (TMR) and multi-nutrient block (MNB); 2) Use of organic and environmentally friendly effective micro-organism (EM) bio-technology; 3) Converting poorly utilized or wasted agro-byproducts; 4) Production and marketing of different kinds of mineral blocks and protein enriched blocks; 5) Piloting new partnership business models; 6) Use of Bentonite as Aflatoxin Binder in commercial feed production; 7) Organizing and conducting practical training in animal feeds and feeding; 8) Disposing social and environmental responsibilities

Ethio-Feed plc employs different market development and promotion strategy. The different livestock feed products are marketed directly to retailers in Adama and the surrounding area and to whole sellers for distribution to distant areas including East Shoa, Arssi, Bale, East and West Hararaghe and Borena Zones of Oromia Region. Afar, Somali Region and Dire Dawa Administration. The feed products are marketed using cash or credit transactions. In areas where there are agro-dealers and farmers service centers having valid license linkage is established by signing agreement for purchase and distribution of the different types of feed products. Commission agents with legal license also distribute the feed on contractual agreement

The preferred types of marketing strategy focus on long term "franchisee business partnership" using innovative business model and approach. This approach will primarily focus on developing joint proposal or business plan that will promote the interest of the two parties and encourage long term joint partnership.

Ethio-Feed in Brief

Ethio-Feed PLC is a commercial feed manufacturing Co., established in 2007 in Adama town by a group of livestock professionals with the objective of producing quality feeds that meet international standards at affordable prices and the vision of producing and availing quality and affordable feed for increased prediction and productivity of the livestock industry in the country and the region.

The mission of the company is using a combination of appropriate inputs, the latest technology and trained human resources, produce quality feed products that meet the expectations of livestock producers. The main key issues that led to the establishment of Ethio-Feed plc are described as follows;

- Low Production and Productivity of Ethiopian livestock. It is frequently told that Ethiopia has huge livestock population ranking 7th in the world and 1st in Africa. However per capita production and consumption (ie milk, meat and egg) is still one of the lowest by sub-Sahara standard. This situation has motivated the company founders to contribute their share to improve production and productivity of the livestock.
- Frequent Drought that kills livestock: since drought is recurring quiet frequently it has brought significant loss to the livestock and in particular to the core breeding livestock particularly in the pastoral and agro-pastoral areas of the country. This calls for intervention in the feed sector to ensure survival and quick recovery of the core breeding stock that are the major source of livelihood to the pastoral and agro-pastoral community as well as source of meat to the domestic and foreign markets.
- Poor health and performance of draft oxen: draft oxen that are used as source of traction in the mixed crop livestock areas of the country are forced to plow farm lands under heavy rain, cold weather and muddy soils. The oxen are provided with dry grass hay or teff straw which is poor nutritionally. Such condition doesn't benefit both the oxen and the farmer. For the oxen to perform better and the farmer to benefit from the farm supplementary feed that contains energy and protein source of feed will be prerequisite.
- Under nutrition of equines (ie horses and donkeys): the most forgotten and mal- treated animals such as horses and donkeys are not given due attention in terms of feed, water and health. To cite an example horse carts that transport humans or goods from pace to place work for about 6-7 hrs/day and are not properly managed and fed. By working continuously for long hours the hoses loose minerals which is not replenished regularly. As result, the horses will worn out and will be left on the streets Therefore, with little amount of mineral supplementation and health care the horse will be healthy and perform better.

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Main Products and Services

- Convert organic agricultural products and byproducts into various quality and affordable feed products.
- Produce quality livestock feed in the form of Total Mixed Ration (TMR), Concentrate, Multi-Nutrient Block (MNB) and Mineral Blocks (MB) for milk, meat, eggs as well as equines and emergency drought response supplementary feed at affordable price.
- Produce quality and affordable poultry feed for starters, pullets, layers and broilers.
- Organize practical training and produce training materials in feeds and feeding to development officers, dairy and feedlot operators, cooperative and union members and pastoral associations.
- Organize and facilitate livestock feed related workshops and seminars.
- Collaborate and partner with likeminded national and international development and research organizations for the growth of the feed industry in particular and livestock industry in general.

What Makes Ethio-Feed PLC Innovative

As commercial livestock feed manufacturing company host of prominent features distinguish Ethio-Feed are the efforts to make the company more attractive and preferable to its customers as presented below.

1. ***Production of quality and affordable total mixed ration (TMR) and multi-nutrient block (MNB)*** with good mix of 13-15 agro-industrial feed ingredients of roughage and concentrate for ruminant livestock (Figure 1). The TMR products are available in 25 kgs and 50 kgs PP bags and the MNBs are available in 1 and 2 Kgs rectangular blocks.

Figure 1. Demonstration of Ethio-feed products such as concentrate mixes and multi-nutrient blocks for visitors,

2. ***Use of organic and environmentally friendly effective micro-organism (EM) bio-technology*** for the production of dairy and fattening livestock products that will increase production and productivity of milk and meat (Figure 2). In addition, its positive contribution it will also improve body condition, production and reproduction. All Ethio-Feed TMR and concentrate feed products are blended with EM

Figure 2. Preparation of effective microorganism (EM) for feed improvement purposes

3. ***Converting poorly utilized or wasted agro-by-products*** such as maize stalk and cobs, sorghum stalks, straws of wheat, barley and teff, passion fruit skin and seeds as well as cactus and prosopis pods into quality and affordable feed products (Figure 3). The products will be properly ground and mixed with commercially available high-quality protein, energy and minerals producing high quality and affordable animal feed products for dairy, and fattening.

Dry maize stalk

Chopped maize stalk

Ground maize stalk mixed with other other feed ingredients producing TMR

Cactus cut and dried | Chopped cactus | Ground cactus mixed with other feed ingredients producing TMR

Figure 3. Feed ingredients and preparation of total mixed rations

4. **Production and marketing of different mineral blocks** (ie high energy, high mineral and high protein) for dairy and fattening animals. Production of mineral blocks (Figure 4) besides reducing importation of the products that saves also foreign exchange, which will enhance capacity of national fed industries' such as Ethio-Feed PLC to diversify its commercial feed products for domestic and export markets in the near future.

Figure 4. Mineral blocks/licks of 1, 2 and 3 kgs

5. **Piloting new "franchisee business partnership model"** with the private sector, youth groups and cooperative unions. Currently, ethio-feed plc has established business partnership with six partners in Ano (Bako area), Dano (Seyo woreda), Wonji (Adama area), Mehon, (Raya Azebo) and Jigjiga town. Locations', feed ingredients available in the respective areas and business partners is presented in Table 1.

Table 1. List of companies and their location which Ethio feed PLC established business partnership.

No	Location	Commodity	Business Partner
1	Wonji, East Shoa, Oromia region	Cane tops, bagasse and molasses	Youth group
2	Ano- Bak, West Shoa, Oromia Region	Maize, Maize stalk, cobs and teff straw	PLC
3	Mehoni, Raya Azebo Woreda, Tigray Region	Cactus, sorghum stalk and teff straw	Coop. Union
4	Seyo Town, Dano Woreda, West Shoa, Oromia Region.	Maize, maize stalk, cobs, noug cake	Youth Group
5	Jigjiga Town, Somali Region	Maize grain, Sesame seed cake, Ground nut cake, Forage leaves.	PLC

There are four advantages in producing different types of animals with the business partners feeds in strategic location. These are:

- ***Reduction in feed production cost by about 10 – 15%:*** availability of some feed ingredients such as crop byproducts as well as oil mill byproducts such as noug, ground nut, rape seed cakes etc close to farming areas in the different geographic locations at relatively cheaper price. Compared to transporting locally available agro-industrial by-products mixed in complete feed ration from central locations in the country where availability and cost of production will be lower by 10-15% if the feed is produced in local area compared to transporting the feed from other commercial feed manufacturing places.

- ***Reduction in transportation cost by 20 - 25%:*** Since most pastoral areas are located far from commercial feed manufacture areas of the central part of the country transport cost of manufactured feed is very high. This implies that high cost of transportation of supplementary feeds including roughages would be too expensive to support livestock emergency feed intervention initiatives. If feed is produced in towns close to emergency areas/town and transported to emergency site there will be cost reduction in transport ranging between 20-25%/qt depending on the location compared to transporting the feed from central parts of the country.

- ***Conservation and utilization of locally available feed ingredients***: feed ingredients such as maize grain, maize stalk and stover, sorghum stalk, cactus as well as molasses, bagasse and cane tops are available in rural setting. In addition, small scale oil seed byproducts such as nug, ground nut and rape seed cakes are also available in rural setting in relatively cheaper price for the production of livestock feed in local areas. In addition, improved forage sources such as Panicum, Sudan grass and Alfalfa are available in rural setting that will enable utilization of locally available feed resources. Using locally available feed sources such as crop byproducts, oil seed byproducts as well as forage and straw/hay different types of animal feed products can be produced in the form of densified forage(straw/stover) based ration), total mixed ration (TMR), block and pellet form. In addition, large scale baled hay or straw formation can be made and stored and fed to animals as source of roughage during dry/drought or normal period. Use of spineless (thorn-less) cactus, Prosopis pods can be widely used in

dry areas, with long term business development initiatives together with other locally available feed sources.

- ***Creating job opportunity for youth groups:*** organized youth in association or cooperatives can be engaged in the supply of the feed ingredients, production, transporting and marketing of the feed products. Even though, there is no generated data or empirical evidence, based on some observation and estimate the following data is generated. Using value chain approach in the production (ie selling of crop byproducts and production of different types of feeds), marketing and transporting feed products it is assumed that 10-12 persons can be employed for 200 - 300 qts. This implies, with production, marketing and transporting of large quantities of different types feed products reasonable number of organized youth groups can be actively engaged and improve their food and income security.

6. **Use of Bentonite as Aflatoxin Binder in commercial feed production -** The issue of aflatoxin in milk has been major concern and discussion point among concerned actors engaged in the dairy industry. At one point the issue has greatly affected the dairy industry to the extent of becoming critical case. This being the fact, oils seed producers, feed ingredient suppliers and traders, commercial feed manufacturers are implicated as source of aflatoxin in feed. Major sources of feed ingredients that have caused aflatoxin in feed are mainly associated with maize and oils seed byproducts such as noug and ground nut cakes. With the aim of minimizing the effect of aflatoxin in milk Ethio-feed plc is using Sodium Bentonite in all its feed products and at present is supplying bentonite to feed manufacturers and livestock owners who are producing their own feed.

7. **Organize and facilitate practical training in animal feeds and feeding** for small, medium and commercial dairy, fattening and poultry owners, development workers, farmers, pastoral and agro-pastoral cooperatives and unions (Figure 5). The training is provided in two forms:

   - Using integrated approach of i) conservation and utilization of agro-industrial byproducts using Effective Micro-Organism with Waljaji Agricultural Industry PLC, ii) forger production and management with Eden Fields Agri-seft PLC and iii) commercial feed production and management by Ethio-Feed PLC. The training is organized in Ethio-Feed Adama production site and will take 7 - 9 days.

   - Commercial feed production and management organized by Ethio-Feed PLC in its Adama production site. The training will take 3 days.

   - In both training the trainees are provided reference training materials and awarded with certificate of participation.

All in all number of trainees in practical commercial feed production and management, conservation and utilization of agro-industrial byproducts using effective micro-organism (EM) and forage production and management ranges between 87 - 95 trainees.

Figure 5. Few of the trainees and participants who are trained and visited Ethio-Feed plc *(Photo: Beruk Yemane)*

8. Contribution to social and environmental responsibilities:

The different innovative approach that will enable the company to undertake social and environmental responsibilities include the following:

Supply and sell of locally available feed ingredients from farm by-products: such as maize stalk and cobs, sorghum stalks, wheat & teff straw and cactus will generate additional income to the farmers. Such arrangement besides increasing availability of feed products will improve accessibility and affordability of feed products to small holder livestock owners. Moreover, the crop byproducts that are left on the ground which will be either wasted or burned before the next planting season that will contaminate the environment will be converted into valuable affordable and quality livestock feed.

The feed that is produced at local level besides generating employment at local level will significantly contribute to profitability of the franchisee partner.

More importantly, allowing permanent staffs to be shareholder in Ethio-feed plc has greatly contributed to develop ownership sentiment and positively resulted in harmonious team building spirit, moral boost and work security among staffs and eventually profitability to the company.

Agro-ecological market niches for Ethio-Feed plc feed products

There are different agro-ecological areas or niches where Ethio-Feed plc has comparative advantages and compatibility for marketing its feed products. The different livestock feed products are sold directly to retailers in Adama and the surrounding area and to whole sellers for distribution to distant areas including East Shoa, Arss,i Bale, East and West Hararaghe and Borena Zones of Oromia Region. In addition, Afar, Somali Region and Dire Dawa Administration are places where there are lot of private, livestock traders and cooperative customers. The feed products are marketed using cash or credit transactions. In areas where there are agro-dealers and farmers service centers with valid license strong linkage and connection is established by signing MoU and contract agreement for purchase and distribution of the different types of feed products. Commission agents with legal license will distribute the feed based on contractual agreement

The preferred types of marketing strategy focus on long term partnership using franchisee business model and approach. This approach will primarily focus on developing joint proposal or business plan that will promote the interest of the two parties and encourage long term joint partnership.

Conclusion

There is general censuses that quality and affordable livestock feed contributes 65- 70% of production cost to milk, meat and eggs. However survival of livestock at times of drought, production and productivity of milk, meat and eggs has been affected, due to various factors. The situation is clearly observed in low production and productivity of the indigenous and cross bred animals, loss of core breeding stock as result of successive drought, poor management and performance of the draft oxen and poor management of equines.

In addition, strong attachment and dependence on conventional commercial feed manufacturing primarily relying on concentrate feed for all types of livestock didn't bring measurable impact on production and productivity. This could be associated with soaring price of concentrate feed ingredients resulting in high price of finished feed or low-quality feed products. At times, shortage of feed ingredients in the market will force commercial feed manufacturers to produce low quality feed that will have no significant effect on production and proactivity.

This being the real scenario, production of livestock feed from other sources such as crop byproducts, high quality forage and mineral sources is not widely practiced. Application of modern and organic bio-technology is not yet practiced. Piloting new business partnership models for production and marketing of commercial feed is at its infancy. Training of livestock owners, development extension officers and livestock marketing individuals and groups as well as feed ingredient suppliers and traders in practical livestock feed production and marketing is not widely practiced.

Moreover, the current commercial feed production and marketing is less gender sensitive and with little environmental concern and rehabilitation work. It has not given due consideration and attention to social concerns and didn't take adequate responsibility to address unemployment and create ideal sentiment for ownership among the permanent working staffs.

In general, productivity of livestock and the commercial feed industry are strongly interrelated and both will show incremental growth and profitability provided that they design and implement "holistic and integrated approach". For any commercial feed company implementation of "***innovative feed solution***" could be one possible solution that will contribute to the profitability and sustenance of the feed industry and growth of the livestock sector.

Recommendations and possible research agenda

Cognizant of the fact that livestock feed plays significant role to the production and productivity of livestock the reality indicates that neither the livestock nor the feed industry has made significant contribution to the development of the sector. This being the fact, the following recommendations are forwarded for researchers to conduct research in the following areas in order to bring the desired change in the feed industry in particular and the livestock sector in general.

New feed product development. It I high time to move from the conventional concentrate feed production and look for diversified forms of feed ingredients and products. These include crop byproducts such as maize stalk, stover, sorghum stalk, straws of teff, wheat, barley, prosopis pods, cactus, molasses, bagasse, cane tops etc. Use of locally available feed ingredients with commercial feed ingredients will enable production of total mixed ration (TMR) which is affordable and quality that can be easily purchased by small holder livestock owners and improve milk and meat production.

The use of green forages as alternative sources of livestock feeds in commercial feeds is very vital. Besides improving the quality of feed for livestock will minimize dependency on grain and oil byproducts concentrate feeds that are very expensive and at times in short supply. This situation could be one research agenda for researchers to look into alternative ways of addressing green forages into commercial feeds.

Drought emergency supplementary feed: at present, feeds that are used as drought supplement are feed sources such as grass, teff or wheat straw, total mixed ration or concentrate feed products transported from the central highland to the drought affected pastoral/agro-pastoral areas of the country. Production of drought supplementary feed that has long shelf life or production of emergency feed from local sources that will minimize production and transport cost has not been widely practiced so far. Use of modern bio-technology such as effective micro-organism that can play significant role in improving the quality of feed and in rumination process has not been given due attention. Therefore, this calls for researchers to dwell in drought emergency livestock feed in particular with due attention to core breeding stock.

1. ***Commercialization of diversified feed products and use of betnonite in feeds***: the use of mineral block/lick is not widely popularized. Blocks produced containing high amount of mineral or protein enriched blocks with significant proportion of green forages sources have not been produced and utilized. Such approach besides reducing dependency on gain

byproducts (ie wheat bran) and oil cakes (ie nug, sesame or ground nut) that are becoming more expensive, will allow shifting to high quality protein, energy, mineral and vitamin sources.

In addition, Aflatoxin in livestock feed has been an issue and use of bentonite in livestock feeds in particular in dairy feed has not been properly addressed. This calls for researchers to make study on different feed products, coupled with animal trail and ultimately check on the effect of using bentonite in minimizing aflatoxin in milk.

2. ***Piloting new partnership business models***: one of the most practiced approach to market livestock feed products is by opening branches. This entails all cost associated with renting house, hiring staff, paying for utilities will be added and paid by the customers. Therefore, as much as possible production and marketing of different types of livestock feeds using different business models such as "franchise business partnership' need to be studied by socio-economists. Such model besides allowing utilization of locally available feed ingredients will also reduce production and transport cost of feed ingredients, enhance local capacity and generate employment for the youth

3. ***Addressing social and environmental responsibility and concerns*** from technical point of view this issue looks bit out of scope. However, in the real case scenario job creation for the youth and rehabilitation of the environment have become more real concerns in the current day of Ethiopia. Therefore, social and environmental researchers in collaboration with commercial feed industries can identify work on research agenda in relation to the above issues to come up with socially and environmentally sound solutions'.

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Yield and Nutritive Value of Different Alfalfa (*Medicago Sativa* L.) Genotypes in Southern Ethiopia

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Abstract

Feed availability and quality is one of the impediments of livestock productivity in Ethiopia. Six alfalfa genotypes were laid out in randomized complete block design with four replications at Arba Minch, Areka, Bonga and Hawassa locations during 2017-2019. Combined analysis of variants revealed that plant height at forage harvest was significantly (P<0.001) varied across tested environments and genotype by environment interaction and dry matter yield significantly (P<0.001) varied for environment variation. Quality parameters such as ash, crude protein and cellulose contents were not significantly (P>0.05) varied among genotypes whereas neutral detergent fiber, acid detergent fiber and lignin, hemicelluloses, in vitro dry matter digestibility and relative feed value were significantly (P<0.01) varied among tested genotypes. The highest mean dry matter yield was recorded for FGI-3054 (2.53 t/ha) followed by FGI-9001 (2.49 t/ha). The highest plant height was recorded for FGI-8091 (75.14 cm), FGI-0916 (65.97 cm), FGI-8091 (71.4 cm) and FGI-1011 (36.95 cm) while the shortest genotypes were FGI-1011, FGI-9001, FGI-5282 and FGI-0916 at Arba Minch, Areka, Bonga and Hawassa, respectively. Crude protein yield was recorded above the threshold level (300.8 to 403.1 g/kg DM) for supplementation of growth and lactation of dairy cattle for all genotypes in the test. Highest in vitro dry matter digestibility (873.6 g/kg DM) and relative feed value (211.2) and lowest neutral detergent fiber (326.9 g/kg DM), acid detergent fiber (192.6 g/kg DM) and lignin (51.3 g/kg DM) and cellulose (141.3 g/kg DM) contents recorded for FGI-9001. Relatively the highest NDF, ADF, ADL, hemicelluloses and cellulose contents recorded for FGI-1011 which was closely similar and above critical values of fiber concentration. Generally, tested alfalfa genotypes varied in terms of forage yield and nutritional quality at different locations indicating the potential for promotion to advanced evaluation and release as better alternatives in the farming system of southern Ethiopia.

Keywords: alfalfa genotypes, crude protein, dry matter yield, plant height, relative feed value

Introduction

Ethiopia has large livestock population (CSA 2015) that contributes 35-49% GDP to agriculture and 37-87% to the household income (Sintayehu et al 2010). Livestock in Ethiopia is estimated to be 183.04 million excluding beehives (CSA 2015). However, feed availability and quality is one of the impediments for proper management and utilization of livestock resources of the country (Yaynshet et al 2016). Feed supply and quality from natural pasture fluctuates following seasonal rainfall dynamics (Solomon et al 2008). Report on feed inventory and feed balances in Ethiopia revealing that it requires 21.2% dry matter, 51.7% metbalizable energy, 48.2% crude protein in quality and 9.5 million-ton

year^{-1} in quantity (FAO 2018). The use of adapted, high yielding, drought tolerant improved forages with high quality are recommended (Mengistu et al 2016).

Alfalfa (*Medicago sativa L.*) is a forage legume having the ability to stay in soil for 3-5 years producing highest economical forage and feeding values among most adapted perennial forage legumes. Root mass formed in very dry condition during the year of establishment of alfalfa (Viliana Vasileva and Ivan Pachev 2015). It is characterized by its ability to tolerate frequent cutting and produce forage every 20-30 days, add nitrogen to soil by bacterial nodules in roots, ability to re-growth after cut and store energy in the crown which helps the buds in a quick re-growth (Abusawar 2004). It uses as pasture, hay, silage, green chop, pellets, soil improvement and soil conservation. It can withstand long periods of water deficit by halting its vegetative growth and accessing water from greater depths through its deeper root system (Annicchiarico et al 2010; Volaire 2008). These properties make alfalfa among one of the most widely grown forage crops in the world. The crop can produce 24 t/h dry matter annually (Brown et al 2000) and it is estimated to be 25% more than pasture (Richard 2011).

Evidences indicated that feeding alfalfa improves productivity of animals; particularly milking cows supplemented with alfalfa could produce over 50-100% additional yield. A practical example is a Horro cow is reported to produce 1.7 liters of milk on average at peak lactation, while 4 liters when fed and managed properly (Tolera 2012). There are various genotypes of alfalfa selected for winter hardiness, drought resistance, tolerance to heavy grazing, pests and diseases (Frame 2005). While selecting alfalfa genotypes in Ethiopia, it is important to focus on the environmental adaptation, herbage dry matter yield potential and seed-bearing ability of candidate cultivars (Geleti et al 2014). This study was therefore designed to identify better yielding and adaptable alfalfa genotype for southern Ethiopia.

Materials and Methods

Description of the study site

The experiment was conducted at Arba Minch, Areka, Hawassa and Bonga Agricultural Research Centers during May 2017 to Feb 2019 under rain fed condition. Arba Minch center is located at $6^0$06'47''N and $37^0$35'10.5''E at an altitude of 1206 metres above sea level. It is characterized with the average monthly rain fall of 53.7 mm, maximum temperature 30-32^{0}C, minimum temperature 16-17^{0}C and relative humidity 53-68%. Texture of a composite soil (0-30 cm) sample collected from the experimental site was a sandy loam, pH 6.2, available phosphorus 14.47, total nitrogen 0.29, organic carbon 1.19 and organic matter 1.63. The pH of experimental soil is within the range for productive soils (FAO 2000). The soils of the experimental site were considered to be medium in available P (Tekalign et al 1991), medium (Landon 1991) to high (Tekalign et al 1991) in N fertility class and in the medium range for organic carbon (Herrera 2005) which is satisfactory for good growth and yield for alfalfa forage. Dubo mante research sub-station, where the experiment was conducted, in Areka agricultural research center which is located at $6^0$4'N, $37^0$41'E and an altitude of 1772 meter above sea level with the mean annual temperature ranged between 6°C and 16°C. The soil of the center is formed from pyroclastic rocks, and is clay in texture (Abayneh, 2003). Dilla is located at $6^0$43'N and $38^0$44'E at the altitude of 1470 meter above sea level. The site is characterized by wet/moist Kolla and highly recognized by its agro-forestry cropping system (Tafesse, 2015). Bonga research center is located at $7^0$19'N, $36^0$13'E and 1723 meter above sea level. Annual average rainfall of the area is 1276 mm and the minimum and maximum temperatures are 9.4^{0}C and 26.02^{0}C, respectively.

Experimental design and treatments

The trial of six genotypes (FGI-8091, FGI-3054, FGI-1011, FGI-5282, FGI-0916 and FGI-9001) were introduced from Forage Genetics International (FGI), USA; the plot were laid out in a randomized complete block design with four replication . The plots size was 4x4m ($16m^2$) and the spacing between rows was 20cm . The seed was was drilled in rows during the main cropping season at all, four locations of the region. All recommended field management practices and recommended seed and fertilizer rate were used.

Data collection

All agronomic (days to 50% flowering; plant height; tiller number per plant), dry matter and nutritive value data were collected. Relative feed value (RFV) was calculated using the formula RFV=DDM (%DM) x DMI (%BW)/1.29 (Uttam et al 2010); where the digestible dry matter (DDM) derived from acid detergent fiber (ADF) and the dry matter intake (DMI) as a percent of the body weight) derived from neutral detergent fiber (NDF). DDM=88.9-(0.779*ADF) and DMI=120/NDF. Hemicellulose and cellulose contents of the genotypes were calculated by the formula neutral detergent fiber (NDF) minus acid detergent fiber (ADF) and ADF minus acid detergent lignin (ADL), respectively.

Statistical analysis

The data generated was statstically analazed using the analysis of variance method and least significance difference, at 5% propability level, in the R software package (R Core Team, 2017).

Results

Combined Analysis of Variance

The combined analysis of variances across locations showed significant differences among the tested genotypes for all measured agronomic traits (Table 1). For all measured agronomic traits, mean square values of genotypes were not significantly (P>0.05) different, whereas for environment were significantly (P<0.05) different. Plant height of alfalfa genotypes were significantly (P<0.05) affected by the interaction effect of genotype by environment. That may indicate consistency of performance of genotypes across tested environments for dry matter yield.

Table 1: Analysis of variance for morphological and yield parameters

Traits	Replication(12)	Genotype (5)	Environment (3)	GxE (15)	Error (60)	Mean	CV%
Plant height (cm)	11.34^{ns}	34.38^{ns}	7259.63^{***}	71.48^{***}	20.66	58.36	7.8
Dry matter yield (t/ha)	1.07^{ns}	0.29^{ns}	16.2^{***}	0.23^{ns}	0.22	2.36	19.8

GxE= genotype by environment interaction, CV%= coefficient of variation

Plant height and forage yield at harvest

Plant height at forage harvesting stage was varied (P<0.05) for genotypes by environment interaction (Table 2). The overall mean plant height was ranged from 56.14 to 60.13 cm with a mean of 58.36 cm. The highest plant height was recorded for genotype FGI-8091(75.14 cm) at Arba Minch whereas the least was FGI-1011. At Areka the tallest plant was FGI-0916 (65.97 cm) while the shortest was

FGI-9001(49.98 cm). The tallest and shortest genotypes at Bonga and Hawassa were FGI-8091 (71.4 cm), FGI-5282 (63.9 cm) and FGI-1011 (36.95 cm) and FGI-0916(30.88 cm), respectively.

Table 2. Mean plant height of Alfalfa genotype in Southern Ethiopia

Genotype	Arba Minch	Areka	Bonga	Hawassa	Mean
FGI-9001	73.83^{ab}	49.98^{f}	69.77^{abcd}	31^{g}	56.14
FGI-3054	71.76^{abc}	62.34^{e}	68.15^{bcde}	35.38^{g}	59.41
FGI-5282	71.43^{abc}	63.24^{e}	63.9^{de}	35.38^{g}	58.49
FGI-8091	75.14^{a}	62.47^{e}	71.4^{abc}	31.5^{g}	60.13
FGI-1011	68.41^{bcde}	52.68^{f}	70.72^{abc}	36.95^{g}	57.19
FGI-0916	71.47^{abc}	65.97^{cde}	67.03^{cde}	30.88^{g}	58.84
Mean	72.01	59.45	68.49	33.51	58.36
LSD	G=3.21	E=2.62	GxE=6.41	CV%=7.8	

LSD=least significant difference, G=genotype, E=environment, GxE=genotype by environment interaction, CV%= coefficient of variation

The highest and consistent plant height was recorded for genotype FGI-8091 followed by FGI-3054 and FGI-0916. The dry matter yield showed non-significant variation among alfalfa genotypes, in all locations (Table 3). The overall mean value was ranging from 2.21 to 2.53 t/ha in which the higher dry matter value was recorded for FGI-3054 followed by FGI-9001. The crude protein and digestible yield showed non-significant (P>0.05) difference among the tested alfalfa genotypes (Table 4). The CP and digestible yields ranged from 0.96 to 1.1 with a mean of 1.03 t/ha and from 2.11 to 2.7 with a mean of 2.64 t/ha respectively.

Table 3. Mean dry matter yield of six alfalfa genotypes in southern Ethiopia.

Genotype	Arba Minch	Areka	Bonga	Hawassa	Mean
FGI-0916	2.64	2.48	2.61	1.10	2.21
FGI-1011	3.00	2.11	2.40	1.40	2.23
FGI-3054	3.29	2.60	2.66	1.58	2.53
FGI-5282	3.29	2.70	2.41	1.03	2.36
FGI-8091	3.21	2.65	2.50	0.92	2.32
FGI-9001	3.70	2.37	2.60	1.31	2.49
Mean	3.19a	2.49b	2.53b	1.22c	2.36
LSD	G=0.33	L=0.27	GxL=0.66	CV%=19.8	

Table 4: Mean of six alfalfa genotypes relative feed value (RFV), crude protein yield (CPY t/ha) and digestible yield (DGY t/ha) in southern Ethiopia

Genotype	CPY	DGY	RFV
FGI-0916	1.10	2.48	193.3^{ab}
FGI-1011	1.03	2.11	159.1^{d}
FGI-3054	1.02	2.60	184.4^{bc}
FGI-5282	1.05	2.70	168.9^{cd}
FGI-8091	0.96	2.65	192.1^{ab}
FGI-9001	1.03	2.37	211.2^{a}
Mean	1.03	2.64	184.85
LSD	NS	NS	22.68
CV%	20.65	16.06	8.15

NS=not significant

Chemical composition of alfalfa genotypes

Neutral detergent fiber, acid detergent fiber, acid detergent lignin, ***in vitro*** dry matter digestibility, relative feed value and hemicelluloses contents were significantly (P<0.05) varied whereas ash, crude protein and cellulose composition were not significantly (P>0.05) varied among genotypes of alfalfa (Table 5). The lowest ADF, NDF and ADL and the highest ***in vitro*** dry matter digestibility (873.6 g/kg DM) and relative feed value (211.24) recorded for FGI-9001. The crude protein content of presented alfalfa genotypes ranged from 300.8 to 400.5 g/kg dry matter with a mean of 369.6 g/kg dry matter.

The IVDMD values of six alfalfa genotypes, which was extending from 751.7 to 873.6 g/kg DM with a mean of 827.1 g/kg DM, presented in table 5 were varied significantly (P<0.01) among each other. The result showed that the highest IVDMD value was recorded for FGI-9001 followed by FGI-8091, FGI-5282 and FGI-1011 while FGI-0916 revealed the least value.

The NDF, ADF and ADL value significantly (P<0.01) varied among six alfalfa genotypes in the present study was in table 5. The values in the present study were ranging from 326.9 to 408.5, 192.6 to 250.2 and 51.3 to 70.5 with mean of 363.3, 224.5 and 59.3 g/kg DM for NDF, ADF and ADL, respectively. The lowest NDF, ADF and ADL content was recorded for FGI-9001 while the highest was for alfalfa genotype FGI-1011.

Table 5: Crude protein, fibers and in *vitro* dry matter digestibility (g/kg DM) of six alfalfa genotypes

Genotype	Ash	CP	NDF	ADF	ADL	IvDMD	Hecell	Cell
FGI_9001	128.9	300.8	326.9^{c}	192.6^{c}	51.3^{d}	873.6^{a}	134.3^{ab}	141.3
FGI_5282	126.5	400.5	389.4^{ab}	236.1^{ab}	65.3^{ab}	839.8^{ab}	153.3^{a}	170.8
FGI_8091	131.7	398.1	349.8^{c}	216.4^{b}	52.9^{cd}	849.5^{ab}	133.5^{ab}	163.4
FGI_1011	122.2	403.1	408.5^{a}	250.2^{a}	70.5^{a}	828.6^{ab}	158.3^{a}	179.7
FGI_3054	130.3	355.1	359.8^{bc}	229.5^{ab}	54.3^{cd}	819.2^{b}	130.3^{ab}	175.2
FGI_0916	132.4	360.1	345.1^{c}	222.4^{b}	61.2^{bc}	751.7^{c}	122.7^{b}	161.2
Mean	128.7	369.6	363.3	224.5	59.3	827.1	138.7	165.3
LSD	NS	NS	39.26	23.53	8.63	46.74	2.9	NS
CV	4.72	13.58	7.17	6.95	9.67	3.75	14.09	7.75

CP=crude protein, NDF= neutral detergent fiber, ADF=acid detergent fiber, IvDMD=in vitro dry matter digestibility, Hecell=hemi-cellulos, cell=cellulose, LSD= leas significant difference, CV%= coefficient of variation, NS= not significant

Discussions

The dry matter yield difference of tested genotypes could be the result of environmental factors such as soil characteristics, moisture conditions, temperature, humidity, pest and disease occurrence and management of the trial field. Environmental factors influencing the growth of crops at various stages of development (Bull et al 1992) due to wider response of genotypes to environment. The consistency of genotypes across environments enables breeders to make effective evaluation of genotypes with minimum cost in a few environments (Gemechu 2012). Selection of better yielding genotypes at one environment may not enable identification of genotypes that can repeat nearly similar performance at another environments (Gezahegn et al 2017).

Genetic variation among genotypes in the trial, response of genotypes to environmental factors and their interactions could be major reasons for the variation of plant height in current study. The significant difference among genotypes for alfalfa plant height was previously reported for different genotypes of Alfalfa and current average result lay in the range of other findings (Wayu and Atsbaha 2019; Gezahegn et al 2017; Wallie et al 2016; Hidosa 2015; Diriba et al 2014).
Other scholars also reported that the dry matter yield was not significantly affected by variety of alfalfa (Wayu and Atsbha 2019). The dry matter yield recorded different value at different locations was for soil acidity, some disease incidence and agro-ecological effect (Akamine et al 2007). The overall mean dry matter yield for 16 cultivars was 12 t/ha (Hayek et al 2008), three cultivars 11 t/ha (Zeinab et al 2013), for five genotypes 6.5 t/ha (Gezahegn et al 2017), 1.78 to 3.32 t/ha (Afsharmanesh 2009), 0.67 to 2.16 t/ha (Awad and Bakeri 2009). Dry matter yield variation of 2.4-2.8; 2.84-4.23; 3.96-4.81; 4.22-4.77; 4.12 and 4.00-4.87 ton ha^{-1} was reported for different alfalfa cultivars (Gashaw et al 2015; Basafa and Taherian 2009; Wayu and Atsbaha 2019; Geleti et al 2014; Befekadu and Yunus 2015; and Walie et al 2016), respectively. The result of present study was lower

than some findings and indeed better than findings of some other scholars. The variation recorded for dry matter yield of different genotypes of alfalfa was for variation in genotypes, environment and their interaction (Gezahegn et al 2017). Growth stage, cut number, leaf to stem ratio, moisture conditions at harvest and processing method are the most important causes of variation for yield of alfalfa (Veronesi et al 2010). Maximum yield on alfalfa is achieved at reproductive maturity when the

The non-significance variation among genotypes for crude protein yield was in agreement with Gezahegn et al (2017) but in disparity for digestible yield. The crude protein as well digestible yield revealed in the present study was lower than the report of Gezahegn et al (2017). The relative feed value (RFV) index was significantly ($P<0.01$) varied among tested genotypes of alfalfa (Table 4). For the present study the RFV value was ranging from 159.1 to 211.2 with the mean of 184.85. The highest RFV value was recorded for FGI-9001 follwed by FGI-0916 and FGI-8091 while the least value recorded for FGI-1011. The RFV reveals the potential intake and fiber digestibility of legumes (Undersander and Moore 2002) and reflect how well an animal will eat and digest a particular forage species when it is fed as the only source of energy (Kazemi *et al*., 2012). The RFV index observed for the cultivars evaluated was higher than a threshold of 151(Redfearn and Zhang 2011) and by far better than the result of Gezahegn et al (2017) while closely similar with Geleti et al (2014) and Mekuanint et al (2015). The feed with higher RFV index of more than 100 considered to be higher quality feed.

Genotypes variation in nutritive chemical composition could be due to harvesting management, genotype and harvesting frequency (Wayu and Atsbaha 2019). The non-significance of alfalfa genotypes reported by other scholars (Wayu and Atsbaha 2019; Gezahegn et al 2017; Mekuanint et al 2015). The highest crude protein value recorded for the genotypes from USA FG sources than from Ethiopia like hairy peruvian agrees with the present result that showed supreme crude protein content when compared with the threshold level (Gezahegn et al 2017; Geleti et al 2014) and USDA standard for high quality (CP>190 g/kg DM) hay making from alfalfa forage for livestock and poultry (www.ams.usda.gov). High quality alfalfa had to contain CP>190 g/kg DM (Kazemi et al. 2012; Redfearn and Zhang 2011) and at full bloom stage it contains CP>160 g/kg DM (Dumham 1998). The crude protein content of all alfalfa genotypes presented in this work was much higher than the indicated threshold value and the reported result of some scholars (Gezahegn et al 2017; Geleti et al 2014; Mekuanint et al 2015; Kaezemi et al 2012). The wider range of values in the literature for crude protein of alfalfa could be attributed to genotypes, climatic and agronomic management practices and/or their interactions (Geleti et al 2014). The genotypes in the present study had a crude protein content of above 150 g/kg DM and it could be suggested for a protein source feed to be considered for use as supplement for lactation and growth in dairy cattle (Geleti et al 2014).

The significant difference among alfalfa genotypes was in agreement with (Geleti et al 2014, Gezahegn et al 2017, Mekuanint et al 2015). The IVDMD reported by other scholars such as 519.5-663.3 (Kamalak et al 2005), 734.8-757.7 (Gezahegn et al 2017), 726.1-795.3 (Wayu and Atsbaha 2019) were lower than those recorded in the present work and in line with the report of Geleti et al (2014) which was ranging from 830.7-837.5 gram per kilogram dry matter. Digestibility of organic matter ranging from 55% to 77% and alfalfa quality depends on soil fertility, cultivar, climatic condition, growth stage, leaf to stem ratio, cutting frequency, harvesting condition, preservation method and processing (INRA 2007; Stancheva et al 2008). Selection for improved forage quality has also been successful for increasing IVDMD in alfalfa (Monirifar 2011).

The significant variation of alfalfa genotypes in NDF and ADF contents was in agreement with previous reports (Geleti et al 2014; Katic et al 2008) due to genetic factors but in disparity with

Gezahegn et al (2017) and Mekuanint et al (2015). High quality alfalfa had to contain the NDF value of <400 g/kg DM and ADF<310 g/kg DM (Redfearn and Zhang 2011; Kazemi et al 2012). On the other hand, according to hay quality designation guidelines of USDA, alfalfa forage quality NDF<440 and ADF<350 g/kg DM fair to supply for domestic livestock use including poultry (www.ams.usda.gov/LPSmarketnews). The NDF, ADF and ADL values of alfalfa genotypes in the present study fell below the critical level and report of some scholars (Geleti et al 2014; Gezahegn et al 2017; Gavan et al 2013; Laura et al 2012; Yu et al 2003; Sheaffer et al 2000; Markovic et al 2007).

The mean value of hemicelluloses significantly (P<0.05) varied among genotypes of presented (Table 5) alfalfa and was higher and cellulose was lower than the reports of Gezahegn et al (2017). Digestibility of alfalfa organic matter depends on the contents of cellulose and lignin. Alfalfa genotypes with lower lignin content have better digestibility due to erective, strong and resistance to plant tissue nature and indigestibility of lignin. A wider range of neutral detergent fiber, acid detergent fiber and lignin of alfalfa genotypes in the present study could be contributed to genetic variation, environmental influence and their interactions.

Conclusion

Feed availability and quality is one of the impediments for proper management and utilization of livestock resources of the country. Among six materials, FGI-9001 genotype found to be better in all parameters in this study. However; all genotypes were recorded high quality according to the standards of the hay making guideline and other reports. Hence, all six genotypes could be competent for quality supply in southern Ethiopia and included in variety registration procedure of the country.

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Spin-Off Technologies From 2nd Generation Biofuel: Potential to Transform Fodder Quality of Crop Residues

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Abstract

The work on 2nd generation biofuels (biofuels based on lignocellulosic biomass rather than on grains as in 1st generation biofuel) has attracted US multi-billion dollars of investment during the last two decades. It may be feasible to utilize spin-offs from 2nd generation biofuel technologies to upgrade lignocellulosic biomass for animal feeding thereby increasing the accessibility of sugars in plant cell walls. Key processes in 2nd generation biofuel that matter for livestock feed resources are: 1) post-harvest collection and mechanical pre-treatment of lignocellulosic biomass; 2) physical-chemical-biological pre-treatment to disrupt lignin-hemicellulose-cellulose matrices, partially hydrolyze weaker linkages of pentoses in hemicellulose structures and make hexoses in cellulose more susceptible to enzymatic hydrolysis; and 3) design and application of targeted and tailored enzyme cocktails. For animal nutritionists, pre-treatment technologies up to the generation of glucose (or equivalents) are interesting, from here on rumen microbes and mammalian enzymes can take over. Three 2nd generation biofuel technologies – 1. Steam treatment, 2. Ammonia Fiber Expansion (AFEX) and Two Chemical Combination Treatment (2CCT) were applied to a wide range of cereal straws and stovers. Increases in in vitro gas production and true in vitro organic matter digestibility (TIVOMD) were greatest upon 2CCT, followed by AFEX and finally steam treatment. Two Chemical Combination Treatment on average increased TIVOMD by 38.2 percentage units from 55.9 in untreated straws and stovers to 94.1% after treatment. When fed to sheep the 2CCT had the greatest effect on livestock productivity promoting an accumulated live weight gain (LWG) after 10 weeks of 6.12 kg which was 3.7 times that of a total mixed ration containing untreated rice straw. Steam explosion treatment was less effective in increasing in vitro digestibility but had a dramatic positive effect on voluntary feed intake, resulting in an organic matter intake of 4% of live weight in male sheep. Applying spin-off technologies from 2nd generation biofuel to upgrading the feeding values of crop residues can transform feed resourcing and feeding. It will not be a farmers' technology but should be embedded into small and medium business enterprises.

Introduction

Lignocellulosic biomass from forest, agricultural wastes and crop residues is the most abundant renewable biomass on earth with a total annual production of about 10 - 50 billion metric tons (Sanchez and Cardena, 2008). About 3.8 billion metric tons are contributed by crop residues with cereals contributing 74%, sugar crops 10%, legumes 8%, tubers 5% and oil crops 3% (Lal, 2005) and crop residues are the single most important livestock feed resources in most low and middle income countries (Blümmel et al., 2014a; Duncan et al., 2016). Cellulose is the major constituent in lignocellulosic biomass ranging from about 300 to 550 g/kg followed by hemicellulose which constitutes about 150 to 350 g/kg and lignin which constitutes about 60 to 300 g/kg (Ivetic and Antov, 2013). Cellulose is a linear polymer of cellobiose which itself is made up of a glucose to glucose dimer in the β 1-4 glucan

configuration. This β 1-4 glucan configuration conveys molecular stability to cellulose when compared to starch, a glucose to glucose dimer in the α 1-4 glucan configuration (Van Soest, 1994). Thus, lignocellulosic biomass is, in its essence, not that different from the primary products of cereals, the starch in grains, even though their respective accessibility to mammalian digestive enzymes is very different (Van Soest, 1994). Considering the huge quantities of lignocellulosic biomass available and the high nutritive quality of their hexose and pentose sugars, it comes as no surprise that attempts to upgrade lignocellulosic biomass for livestock fodder reach back to the beginning of the 20th century (Fingerling and Schmidt, 1919; Beckmann, 1921).

The work on 2nd generation biofuels (biofuels derived from lignocellulosic biomass) was motivated by reasons very similar to those of the early animal nutritionists: the abundance of lignocellulosic biomass and its content of basic sugars. The work on 2nd generation biofuels has attracted US multi-billion dollars of investment during the last two decades (Blümmel et al., 2014b). It may be feasible to utilize spin-offs from 2nd generation biofuel technologies to upgrade lignocellulosic biomass for animal feeding thereby increasing the accessibility of sugars in plant cell walls. The current paper explores the impact of three

2nd generation biofuel technologies on the fodder quality of a wide range of cereal straws and stovers: 1) Steam explosion treatment; 2) Ammonia Fiber Expansion (AFEX); and 3) Two Chemical Combination Treatment (2CCT).

Materials and Methods

Steam explosion treatment: - For laboratory analysis maize stover from a superior dual-purpose hybrid, a superior dual purpose sorghum variety and two sorghum stovers purchased from the fodder market were steam-treated using intermittent live steam injection to heat stovers to 160°C for 10 minutes. After 10 min the stovers were exploded into a receiver tank and dried (Dhanalakshmi et al., 2015). For sheep feeding trials rice straw from variety MTU 1010 was purchased and steam explosion treated in an analogous fashion.

Ammonia Fiber Expansion (AFEX): - This technique was developed by Dale and Weaver (2000). During AFEX treatment, ammonia vapor is added to the biomass under moderate pressure (100 to 400 psi) and temperature (70 to 200°C) before rapidly releasing the pressure and recovering more than 95% of the ammonia used in the process. For laboratory analysis ten cereal straws and stovers from India consisting of two rice straws, three sorghum stovers, one wheat straw, two pearl millet stovers and two maize stovers were treated. (AFEX is a Trademark and no larger amounts of rice straw could be treated for feeding trials)

Indian Institute for Chemical Technology (IICT) 2 Chemical Combination Treatment (2CCT)

This technology has been developed by the Indian Institute for Chemical Technology (IICT) for biofuel production and is currently being prepared as a joint CSR-IICT and ILRI patent application. The approach is therefore only described as 2 Chemical Combination Treatment (2CCT). For laboratory analysis four pearl millet stover, three sorghum stover, two maize stover and two were treated. For sheep feeding trials rice straw from variety MTU 1010 was purchased and steam explosion treated in an analogous fashion.

Laboratory analysis: - Nitrogen was analyzed by the Kjeldahl method. Neutral (NDF) and acid (ADF) detergent fibre and acid detergent lignin (ADL) were analyzed according to Van Soest et al. (1991). In vitro apparent digestibility and ME content were calculated from in vitro gas production after 24 h using the equations of Menke and Steingass (1988) but with the in vitro incubation procedure modified according to Blümmel and Ørskov (1993). True In vitro organic matter digestibilities (TIVOMD) after 24 and 48 h were analysed according to Goering and Van Soest (1970) by refluxing of incubation residues from the gas syringes as described by Blümmel and Ørskov (1993) with neutral detergent solution.

Sheep feeding trials: - Three is nitrogenous total mixed rations (TMR) each consisting of about 70% untreated, 2CCT and steam explosion treated rice straw were designed and each fed to six sheep housed in metabolic cages. The TMR were fed ad libitum allowing for about 10 to 15% of refusals. Measured were daily intake and liveweight changes.

Results

Comparison of effectiveness of steam explosion, AFEX and 2CCT treatment on in vitro measurements

Increases in in vitro gas production (GP) and true IVOMD measured after 48 h of incubation were greatest upon 2CCT followed by AFEX and finally steam treatment (Table 1). The increases in in vitro GP were 10, 20 and 68% upon steam explosion, AFEX and 2CCT treatment, respectively. TIVOMD increased by 14, 30 and 68% respectively upon steam explosion, AFEX and 2CCT treatment, respectively. In absolute terms after 48 hr of incubation AFEX and 2CCT on average increased true IVOMD by 19.3 (65.1 to 84.4%) and 38.2 (55.9 to 94.1%) percentage units, respectively.

Table 1. Summary of effects of steam, ammonia fiber expansion and 2CC treatment on in vitro gas production (GP) and true in vitro organic matter digestibility[-1] after 48 h of incubation. U = untreated; T = Treated

Spin-off technology		In vitro GP after 48 h (ml/200 mg)		True IVOMD after 48 h (%)	
	n	U	T	U	T
Steam Treatment	4	48.6	53.6	62.9	71.8
AFEX Treatment	10	42.9	51.5	65.1	84.4
2CC Treatment	11	39.7	66.7	55.9	94.1

[-1] The average difference between true and apparent IVOMD is about 12.9 percentage units (van Soest, 94).

Effectiveness of steam explosion and 2CCT treatment on rice straw in total mixed rations (TMR) on laboratory fodder quality traits

Organic matter, CP, NDF, ADF, ADL, in vitro GP and TIVOMD measured after 24 and 48 hr in the TMRs consisting of about 70% of untreated and two chemical combination (2CCT) and steam explosion (SE) treated rice straw are reported in Table 2. The TMRs were close to isonitrogenous. NDF, ADF and ADL seem unsuitable laboratory analytical techniques for testing effects of 2CCT and steam explosion treatments. For example, 2CCT treatment could increase NDF and ADF content over that of untreated TMR while steam explosion treatment increased the recovery of ADF over NDF and almost doubled the ADL content relative to untreated TMR.

The in vitro GP and TIVOMD responded to treatments in that GP more than doubled when compared after 24 hr of incubation and was 84% higher when compared after 48 hr in 2CCT TMR relative to control TMR (Table 2). TIVOMDs also increased by 50 and 40% after 24 and 48 hr over control TMR, respectively reaching more than 90% of TIVOMD. Steam explosion treatment resulted in 23 and 12% higher GP after 24 and 48 hr, respectively, relative to control TMR while TIVOMD was 28 and 21% higher after 24 and 48 hr, respectively.

Table 2. Organic matter (OM), crude protein (CP), neutral (NDF) and acid detergent fiber (ADF), acid detergent lignin (ADL) in vitro gas production (GP) and true in vitro organic matter digestibility (TIVOMD) measured after 24 and 48 hr in total mixed rations (TMR) consisting of about 70% of untreated and two chemical combination (2CCT) and steam explosion (SE) treated rice straw

Treatment	OM	CP	NDF	ADF	ADL	GP24	GP48	TIVOMD24	TIVOMD48
	(%)	(%)	(%)	(%)	(%)	(ml)	(ml)	(%)	(%)
Untreated	81.7	12.7	56.6	43.7	3.7	27.9	37.7	60.7	68
2CCT	85.8	12.4	63.6	53.2	2.1	58.4	69.3	90.8	94.7
SE	80.1	12.7	41.1	47.4	6.1	34.4	42.3	77.9	82.5
P > F	<0.0001	0.5	<0.0001	0.002	0.0001	0.0001	<0.0001	<0.0001	0.0001
LSD	0.8		23	35	0.91	6.4	5.7	2.4	5.9

Effectiveness of steam explosion and 2CCT treatment on rice straw in total mixed rations (TMR) fed to sheep

The responses of sheep fed TMR consisting of about 70% untreated, steam explosion and 2CCT treated rice straw are reported in Figure 1. The 2CCT treatment had the greatest effect on livestock productivity promoting an accumulated live weight gain (LWG) of 7.85 kg, which is 3.4 times that of the TMR containing untreated rice straw. While steam treatment was less effective in increasing in vitro digestibility than 2CCT (Table 1) its positive effect on voluntary feed intake was found to be dramatic, resulting in an organic matter intake of about 4% of live weight in male sheep! This very high intake promoted accumulating LWG of 6.28 kg., which is 2.7 times that of sheep fed TMR with untreated rice straw.

Discussion

Urgency of increasing fodder quality of crop residues such as straws and stover

Feed, both shortage and costs, acts as a major constraint to higher livestock yields; this feed constraint will become more binding with the increasing demand for animal sourced food (ASF). Opportunities for improving feed resources are constrained by shortages of arable land and increasingly water; and these constraints are likely to become aggravated by climate change (Blümmel et al., 2015). Feed supply-demand scenarios for South Asia and East and West Africa have shown that crop residues such as straws, stover and haulms are generally the single most important feed resources often providing between 50 and 70% of the feed resources in small holder systems (Blümmel et al., 2014a; Duncan et al. 2016). Crop residues are considered to be of low fodder quality, though this is essentially only true for cereal crop residues since leguminous residues can have excellent fodder quality. Even the poor fodder quality of cereal crop residues is not because of an inherent low nutrient content but due to the molecular formation of its basic sugars. In ligno-cellulose, glucose to glucose dimers are in the β 1-4 glucan configuration and this β 1-4 glucan configuration conveys molecular stability to ligno-cellulose when compared to starch, a glucose to glucose dimer in the α 1-4 glucan configuration (Van Soest, 1994). Thus, lignocellulosic biomass is, in its essence, not that different from the primary products of cereals, the starch in grains, even though their respective accessibility to mammalian digestive enzymes is very different (Van Soest, 1994). The high content of basic sugars of ligno-cellulose biomass of course prompted the 2nd generation biofuel work with the aim of converting ligno-cellulose biomass into Ethanol. Blümmel et al. (2014b) argued that technologies developed for converting ligno-cellulose biomass into ethanol could be useful in animal nutrition to make basic sugars in lignified plant cell walls more accessible to rumen microbes and even mammalian enzymes.

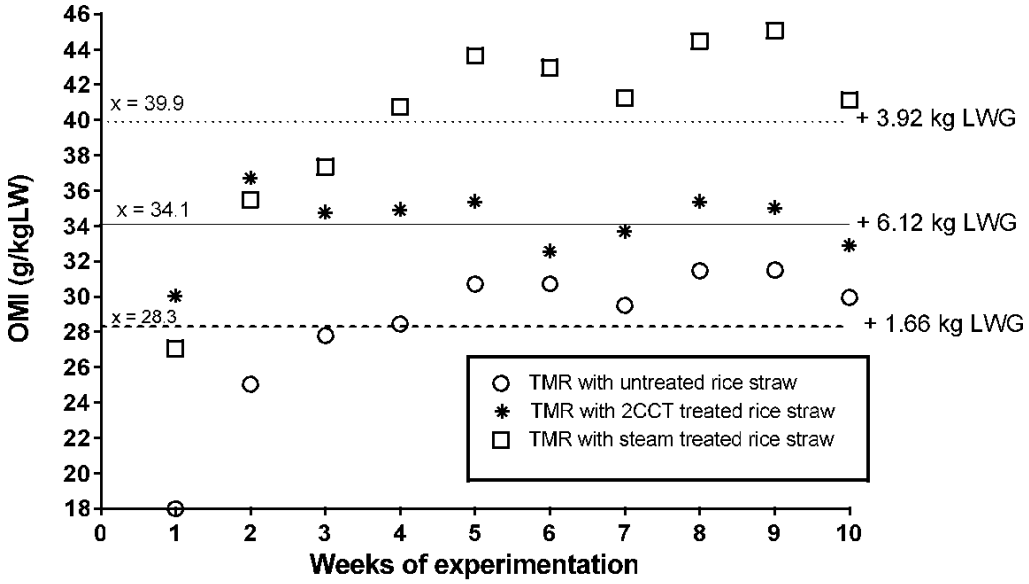

Figure 1: Response of sheep fed total mixed rations containing 70% of untreated, 2CCT treated and steam explosion treated rice straw

Effectiveness of steam explosion, AFEX and 2CCT treatment on laboratory fodder quality traits

The current work investigated three 2nd generation biofuel technologies, steam explosion, AFEX and 2CCT. These three technologies are in order of increasing complexity in that steam explosion treatment is based only on water and pressure (Dhanalakshmi et al., 2015), AFEX on one chemical (ammonia) and mild pressure (Dale and Weaver, 2000) and 2CCT (Blümmel et al., 2018) on two chemicals, one pH changing and the other one oxidative. Increases in in vitro GP and TIVOMD measured after 48 h of incubation were greatest upon 2CCT, followed by AFEX and finally steam explosion treatment (Table 1). The increases in in vitro GP were 10, 20 and 68% upon steam explosion, AFEX and 2CCT treatment, respectively, while TIVOMD increased by 14, 30 and 68% upon steam explosion, AFEX and 2CCT treatments, respectively (Table 1). In absolute terms increments of TIVOMD upon steam, AFEX and 2CCT treatments were 8.9, 19.3 and 38.2 percentage units, respectively (Table 1). Particularly AFEX and 2CCT treatments will have potentially very significant impacts on fodder quality of cereal straws and stovers if these TIVOMDs can be translated into animal performance, in effect turning cereal crop residues into concentrates (Table 1).

The TIVOMD measurement is gravimetric in nature and calculated from the truly undegraded residue; all substrate not recovered is supposed to have been fermented. This might not always be the case particularly in treated feed stuffs where some unfermentable substrate might have been solubilized and so not recovered in the incubation residue (Blümmel et al., 2005). There is some indication that this might have happened upon steam explosion and AFEX treatments where percentage increases in in vitro GP were less than increases in TIVOMD. In 2CCT, however, the increase in TIVOMD of 68% agreed with the average increase in vitro GP of 66% (66.7 vs 39.7 ml) and in vitro GP reflects generation of fermentation products and so is not gravimetric in nature. Put differently, the tremendous increases in straws and stover quality upon 2CCT treatment seem real.

Effectiveness of steam explosion and 2CCT treatment on sheep performance

The effectiveness of steam explosion and 2CCT treatments were not restricted to laboratory fodder quality traits, as shown by feeding of TMRs with untreated and treated rice straw to sheep. The responses of sheep fed TMR consisting of about 70% untreated, steam explosion and 2CCT treated rice straw are reported in Figure 1. The 2CCT treatment had the greatest effect on livestock productivity promoting an accumulated live weight gain (LWG) 7.85 kg which is 3.4 times that of the TMR containing untreated rice straw. While steam treatment was less effective in increasing in vitro digestibility than 2CCT (Table 2) its positive effect on voluntary feed intake was found to be dramatic, resulting in an organic matter intake of about 4% of live weight in male sheep. This very high intake promoted accumulating LWG of 6.28 kg, which is 2.7 times that of sheep fed TMR with untreated rice straw.

Conclusions

Spin- off technologies from 2nd generation biofuels have considerable potential for transforming the fodder quality of crop residues. Animal nutritionists can, and should, leverage from these multi-billion investments into 2nd generation biofuel in collaboration with the private sector. Small and medium enterprises applying these spin-off technologies would: 1) very significantly increase fodder quality of crop residues thereby increasing livestock productivity at decreased feed costs; and 2) generate employment and income opportunities for rural population; and 3) decrease environmental hazards from localized burning of crop residues (mainly rice straw). We also want to stress that the three pre-

treatments explored are not exhaustive and other potentially useful spin-off technology from 2nd generation biofuel are out there and need to be investigated

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Effect of Climate Change on Global Corn Production: Impact on Corn Silage Production and Ensiled Italian ryegrass (*Lolium multiflorum Lam.*) and Winter Cereal Mixtures as Alternative Options

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Abstract

Nowadays climate change is altering crop cultivation practices in many areas of the world. Recent research outputs and reports were reviewed to address the core issue of climate change effect on global corn production and corn silage production. Additionally, an experimental study report of nutrient composition and fermentation characteristics of ensiled Italian ryegrass (Lolium multiflorum Lam.) and winter cereals mixtures were presented for comparison. Results of current research findings reveal that sever climatic conditions such as drought, high summer heat waves, ground water shortage and mycotoxin contamination increase the risk of corn crop failures across the globe. Climate change together with the accelerated use of corn in bioethanol production mainly in US, as well as global use of corn as human food have substantially affected the mass production of corn. Study report on sensitivity of corn silage to climate change reveals that, management practices in relation to corn silage production governed by the current climate and local conditions must change considerably by the warming climate. Thus, it would be urgent to consider how crop production and livestock feeding strategies can be adapted to this change in long-term taking into account the nutrient requirements of high producing lactating cows. Accordingly, interest in new alternative forages has increased in recent years even though reports are not frequent. A new study report reveals that when Italian ryegrass and winter cereals ensiled together, it produces silage with good nutritional composition and fermentation quality compared to other grass and cereal silages ensiled alone. Efficient fermentable characteristics of ensiled mixtures with excellent fermentation end products like high lactic acid (>75% of total fermentable acid) with very low NH_3 – N (< 0.9 gkg^{-1}Tot N), ethanol (< 0.2% DM) and acetic acid (<2.5% DM) together with better nutrient recovery such as higher CP (15.2 and 16.1% DM) and lower NDF (481 and 500 gkg^{-1} DM) and ADF (320 and 337 gkg^{-1} DM) fraction could make the mixture silages as viable alternative forage to at least partly replace corn silage.

Key words – climate change, corn silage, Italian ryegrass, winter cereals

Introduction

According to the World Metrological Organization (2018) report, the 2017 global mean temperatures were about 1.1 °C above pre-industrial temperatures. The global temperature of the five-year average (2013–2017), is the highest five-year average on record. The world's nine warmest years have all occurred since 2005, and the five warmest since 2010. The report further states that, the start of 2018 had continued with extreme weather unusually high temperatures, bitter cold and damaging winter storms, extreme heat waves, continued drought and acute water shortages across the globe. In Europe, since the early 1900s, an increase of about 0.9 °C in temperature has been experienced (Ozturk et al.,

2018). These changes in climatic conditions currently altering crop cultivation practices in many areas of the world (Ozturk et al., 2018) particularly those crops produce at a global maximum level such as corn. In this regard very recent study report reveals that, climate change will increase the risk of corn crop failures across the world's biggest corn-growing regions (Tigchelaar et al., 2018). According to the report (Table 1), an increase of 4°C close to where the current greenhouse gas emissions trajectory, could cut U.S. corn production by nearly half at the end of the century. But, if global warming is instead held to 2°C (the goal of the Paris climate agreement is to stay below that level) the projected loss in U.S. corn production would be closer to 18%.

Table 1. Projected changes in total production for each of the top-four maize producing countries in response to a 2°C and 4°C warming.

Country	**2ºC warming**	**4ºC warming**
USA	- 17.8%	- 46.5%
China	- 10.4%	- 27.4%
Brazil	- 7.9%	- 19.4%
Argentina	- 11.6%	- 28.5%

Source: Tigchelaar et al., 2018

However, maintaining the global warming below 2°C could not be possible according to the recent report of the United Nations (2018) intergovernmental panel on climate change. This report states that, global warming is likely to reach 1.5°C between 2030 and 2052 if it continues to increase at the current rate and even many of the adverse impacts of climate change will come at the 1.5°C mark even though previous estimates focused on estimating the damage if average temperatures were to rise by 2°C. Tigchelaar et al. (2018) reported that, much of the world's corn production goes into livestock feeding and making biofuels implies how livestock sector is still dependent on corn production. Corn crop failure associated with climate change effect and other interrelated factors such as competition with human and other livestock, massive use of corn for bioethanol production will make the fate of the future dairy industry more complicated particularly intensive corn silage dependent dairy farming's in Europe and Northern America. Therefore, finding and robust application of alternative forage crops to at least partly replace corn silage is considered absolutely necessary to the success of future dairy industries. This study was aimed to address effect of climate change on global corn production, its impact on corn silage production and different viable options to mitigate corn crop failure associated with climate change effect. Additionally recent finding of ensiled Italian ryegrass (*Lolium multiflorum Lam.*) and winter cereals mixtures were presented for comparison.

Materials and Methods

Recent research outputs, conference proceedings and panel discussion reports were reviewed to address the core issue of climate change on global corn production, its effect on corn silage production and viable alternative options. Additionally, an experimental study report of nutrient composition and fermentation characteristics of ensiled Italian ryegrass (*Lolium multiflorum Lam.*) and winter cereals mixtures were included for comparison.

Results and Discussion

a) Climate change effect on corn silage production and viable alternative options

Corn silage is the main forage fed to dairy cows in Europe and North America. However, in recent years, difficulty occurs in corn cultivation due to climate conditions such as drought, high summer heat waves, ground water shortage, mycotoxin contamination (Salomone et al., 2013; Kálmán and Rajki, 2015; Getachew et al., 2016; Tigchelaar et al., 2018; and David and Gary, 2018). Climate change together with the accelerated growth in ethanol production mainly in US (USDA, 2012; David and Gary, 2018) as well as global use of corn as human food for around 800 million people living in extreme poverty (Tigchelaar et al., 2018), have substantially affects the mass production of corn and make the fate of future dairy industry more complicated. As corn production globally affected by climate change, the effect is similar for forage corn production for silage making. In this regard very recent report by Ozturk et al. (2018) on sensitivity of silage corn to climate change reveals that, management practices in relation to silage corn production governed by the current climate and local conditions must change considerably by the warming climate. Thus it would be urgent to consider how crop production and feeding strategies can be adapted to this change in long term taking into account the nutrient requirements of high producing lactating dairy cows. So far tremendous effort had been exerted to at least minimize climate induced effect on forage corn production for silage making. As per our review and what scientists were suggested, the following three possible options could partly resolve the anticipated and unavoidable future climate change effect on forage corn production for silage making depending on the suitability, sustainability, availability, cost and accessibility of the technology.

I. *Intensive use of irrigation to boost forage corn production for silage making*

Intensive use of irrigation mainly drip, sprinkler and sub surface irrigation to boost forage corn production has still been practicing in different parts of the world depends on the availability, quality, cost and accessibility of water. So far some promising results were reported regarding the use of irrigation to boost forage corn production (Lamm and Trooien, 2003; Simsek *et al.*, 2011; Karasahin, 2014; Karasu *et al*., 2015, Kisekka *et al*., 2016; Nilahyane *et al.*, 2018; Lopresti and Bertin, 2018). However, Tigchelaar *et al*. (2018) argued that even the supply of and demand for irrigation water now a days severely influenced by climate change. On the other hand cost of irrigation is not affordable under farmer's level in many countries particularly in developing countries. In some countries irrigation is not possible at all due to either shortage of suitable water or due to legislative restrictions on the use of water for irrigation (Van Duinkerken et al., 1999). For instance, in Hungary arable crops are not irrigated (Kálmán and Rajki, 2015). Thus, there are some limitations in the use of irrigation to boost forage corn production. As a result irrigation may have limitation to be used as an alternative option in the future but still an ideal option if there is conducive situation to use it.

II. *Genetic approaches to improve corn forage yield, nutritional quality & digestibility*

In recent years corn forage hybrids have been developed specifically to replace the conventional corn forages fed to livestock (Roth and Heinrich, 2001; Kung, 2011; Grant and Contanch, 2012; Arriola et al., 2012; Dewhurst, 2013; Salomone et al., 2013; Ferraretto et al., 2015). The development of corn hybrids played important part in the worldwide success of corn silage, and the choice of a suitable hybrid is the most important factor for profitable silage production. Plant breeders have made considerable advances in achieving earlier maturing corn varieties that are more reliable for a specific area (Dewhurst, 2013). However despite tremendous improvements in forage corn breeding and crop yield potential, production of hybrid forage corn remains highly dependent on climate (Lathrop and

Namuth, 2011). This is attributed to solar radiation, temperature, and precipitation are the main drivers of crop growth (Ozturk et al., 2018; Tigchelaar et al., 2018). In addition, corn plant diseases and its genetic resistance (Hurni et al., 2015; Mubeen et al., 2017; Caliano and Miedaner, 2017; Burns, 2017) as well as frequent outbreak of pest infestations such as the fall army worm (Niassy and Subramanian, 2018) following harsh climatic condition are currently great threat for a new forage corn hybrids. Due to those facts, genetic approach would not be possible in the future despite drought tolerance corn hybrids are currently used in areas where water shortage is a problem (Lathrop and Namuth, 2011). The authors further warn that, eventhough drought tolerant corn offers some advantages, care should be taken to determine whether these particular hybrids are suitable for certain farming operation. Drought tolerant corn does not offer a yield advantage over other hybrids under irrigated or rain fed conditions according to Lathrop and Namuth, (2011) report. This is because drought tolerant corn, simply performs better than other hybrids under limited-water conditions.

III. ***Identifying alternative forage crops to fill intensive silage demands prone to corn crop failure.***

The third possible option, which has been recently given attention is identifying viable alternative forage crops Use of alternative fodder crops considering the yield safety of corn silage might be compromised in the future if the expected climate change characterized by the increase summer heat waves and more extreme water course. However, due to its excellent nutritional profile, high dry matter and energy content, higher organic matter digestibility and improve milk production ability, it could be difficult to replace whole crop corn silage with other forage particularly for high producing dairy cows. In this regard, for the last 20 years different scientists attempted to replace whole crop corn silage with other alternative fodder crops silage such as whole crop triticale silage (Van Duinkerken et al., 1999), annual ryegrass silage (Bernand et al., 2002), whole crop rice silage (Ki et al., 2009), Italian ryegrass silage (Baldingar et al., 2012; 2014), different forage millet cultivars silage (Brunette et al., 2014), lucerne silage (Sinclair et al., 2015), whole crop sorghum silage (Colombini et al., 2015, Cattani et al., 2017, Khosrani et al., 2018), high sugar forage sorghum silage (Su-jiange et al., 2016) and wheat and triticale silage (Harper et al., 2017) considering the global climate change effect on yield and safety of corn production for silage making. However, all the studies reported limitations in their attempts to replace whole crop corn silage. Unsatisfactory result was reported by both Brunette et al. (2014) and Sinclair et al. (2015) in their attempt to replace different forage millet silage cultivars and lucerne silage with whole crop corn silage respectively. Cows fed corn silage performed better than those fed regular millet or sweet millet likely due to the higher starch and lower NDF intakes (Brunette et al., 2014). On the other hand Sinclair et al. (2015) reported that, a high inclusion rate of lucerne silage is associated with a reduction in digestibility, and increased plasma concentrations of 3-OHB and urea. A mild to relatively very good result was reported by Ki et al. (2009), Cattani et al. (2017) and Khosrani et al. (2018) in their attempt to replace whole crop rice and sorghum silages with corn silage. Ki et al. (2009) reported that, whole crop corn silage can be replaced with whole crop rice silage in the diets of mid to late lactating Holstein cows without any deleterious effects on feed consumption, milk yield and its composition. The substitution of corn silage with sorghum silage did not change the concentration of saturated and monounsaturated fatty acids, but reduced the concentration of polyunsaturated fatty acids in milk (Cattani et al., 2017). In addition, n-6 and n-3 fatty acids resulted lower in cow's milk fed sorghum diet as compare to cows fed corn diet. They further noted that milk coagulation properties, which have a great economic relevance for the Italian dairy industry, were not altered by the substitution of the corn silage with the sorghum silage in dairy cows. These preliminary results suggest that forage sorghum silages could have a potential as substitute of corn silages in dairy cow diets. On the other hand Khosrani et al. (2018) reported that, sorghum silage can be fed to lactating Holstein cows as a total replacement for corn silage without undesirable effects on animal performance, but with positive effects

on antioxidant capacity and polyunsaturated fatty acids of milk. They further conclude that forage sorghum silage can be an excellent choice for dairy farms in areas where cultivation of corn is difficult. However, an excellent and relatively promising result was reported by Van Duinkerken et al. (1999), Bernand et al. (2002), Baldingar et al. (2012, 2014), Harper et al. (2017) in an attempt to replace corn silage with Italian ryegrass, annual ryegrass and winter cereals (wheat and triticale) silages. Van Duinkerken et al. (1999) reported that there is no significant difference in either feed intake or lactation performance between triticale whole crop silage and corn silage. However, calculated the intake of net energy for lactation was lower at the triticale based ration. The author concluded that, the net energy value for lactation is underestimated for triticale whole crop silage. Harper et al. (2017) noted that, at milk production of around 42 kg/d, wheat silage and triticale silage can partially replace corn silage without affecting DM intake, but milk yield may slightly decrease. For dairy farms in need of more forage, triticale or wheat double cropped with corn silage may be an appropriate cropping strategy. On the other hand Bernand et al. (2002) reported that, substituting ryegrass silage for a portion or all of the corn silage in diets fed to lactating dairy cows can improve yield of milk and components. Baldingar et al. (2012) reported that inclusion of Italian ryegrass silage in the diet increased forage intake significantly (14.5 vs 13.4 kg DM in the control group) and concentrate intake did not differ, but milk yield was slightly lower (20.3 vs 21.0 kg) owing to the low energy and protein concentration of Italian ryegrass silage. However they further reported that Italian ryegrass was indeed found to be highly palatable, confirming in principle its suitability as feed for organic dairy cows. Additionally, higher energy and protein concentrations in Italian ryegrass forage would be necessary to translate the high intakes of Italian ryegrass silage into improved milk production as well.

b) Ensiled Italian ryegrass and winter cereal mixtures as viable alternative options

Italian ryegrass (*Lolium multiflorum*) is one of the fast growing grass species with high forage yields, high fiber digestibility (NDFD), high crude protein and sugar content, palatability, resistance to winter hardiness, ease of establishment, high yield response to nitrogen and suitable for silage making (Baldingar et al., 2012,2014; Bagg, 2014; DLF seeds, UK, 2018; Byron Seeds, LLC, 2019). However Bagg, (2014) reported that the yield of Italian ryegrass is not as high as winter cereals such as oats, but nutrient quality and palatability is greater which makes it more suitable for high producing dairy cow feed. High DM and Energy content, outstanding fermentable characteristics and superior intake and organic matter digestibility are among the many reasons difficult to replace whole crop corn silage with other crop silages. However recent study (Alemayehu et al., 2019) reveals that, when Italian ryegrass and winter cereals ensiled together, it produces silage with an excellent nutritional composition (Table 2 and 3) as compared to the nutritional composition of grass, cereal silages and rye grass silage ensiled alone. The fermentation quality of ensiled mixtures forage was also good (Table 4).

Table 2. Nutritional composition of two different mixtures of Italian ryegrass and winter cereal silage

(% DM)	DM	CP	EE	CF	Ash	NDF	ADF	ADL	Sugar	NFE
Mixture A*	27.46	15.24	4.00	27.06	15.58	48.1	32.04	9.82	0.12	38.12
Mixture B**	31.34	16.08	4.20	28.60	13.14	50.36	33.72	4.46	0.04	37.98

** Mixture A (three types Italian ryegrass 40% + two types triticale 20%+ two types of oats 20%+ wheat 15%+ barley 5%), ** Mixture B (three types Italian ryegrass 55% + two types winter oats 45%),*
DM – dry matter, CP – crude protein, EE – ether extract, CF – crude fat, NDF – neutral detergent fiber, ADF acid detergent fiber, ADL – acid detergent lignin, NFE – nitrogen free extract, Source: Alemayehu et al., 2019

Even though compared to corn silage the DM, NDF and ADF contents of Italian ryegrass and winter cereals mixture silages are lower (Table 3), the high CP value together with lower NDF and ADF fraction would greatly improve the intake, rumen degradability and over all tract digestibility of mixed silage forage. This make the ensiled mixtures are alternative to replace corn silage

Table 3. Nutritional composition of silages made from pure and mixture of different forages

Silage type	DM (%)	CP (%)	EE (%)	NDF (%)	ADF (%)	ADL (%)	Ash (%)	Sources
Mixture A*	27.46	15.24	4.0	48.10	32.04	9.82	15.58	Alemayehu et al.(2019)
Mixture B**	31.34	16.08	4.2	50.36	33.72	4.46	13.14	Alemayehu et al.(2019)
Corn silage	35.10	8.80	3.2	45.00	28.10	2.60	4.30	NRC, 2001
Rye grass silage	36.50	12.80	3.1	60.70	40.30	6.90	8.10	NRC, 2001
Sorghum silage	28.80	9.10	2.9	60.70	38.70	6.50	7.50	NRC, 2001
Barely silage	35.50	12.00	3.5	56.30	34.50	5.60	7.50	NRC, 2001
Oats silage	34.60	12.90	3.4	60.60	38.90	5.50	9.80	NRC, 2001
Triticale silage	32.00	13.80	3.8	59.70	39.60	5.80	9.70	NRC, 2001
Wheat silage	33.30	12.00	3.2	59.90	37.60	5.80	8.60	NRC, 2001

* Mixture A (three types Italian ryegrass 40% + two types triticale 20%+ two types oats 20%+ wheat 15%+ barley 5%),
** Mixture B (three types Italian ryegrass 55% + two types winter oats 45%), DM – dry matter, CP – crude protein, EE – ether extract, NDF – neutral detergent fiber, ADF – acid detergent fiber, ADL – acid detergent lignin

Table 4. Fermentation quality of mixtures of Italian rye grass and winter cereals, and corn silage alone.

Attributes	Mixture A*	Mixture B*	Corn silage**
NH3-N (g/kg Tot N)	0.88	0.81	0.80 - 1.12
pH	4.26	4.39	3.7 - 4.2
Ethanol (%DM)	0.16	0.11	1-3
Acetic Acid (%DM)	2.25	1.91	1-3
Butyric acid (%DM)	0	0	0
Lactic acid (%DM)	8.92	7.03	4-7
LA/AA	3.97	3.67	2.75
VFA (%TFA)	20.16	21.41	30 - 35
LA/%TFA	79.84	78.59	65 - 70

** Alemayehu et al, 2019, ** Kung and Shaver (2001), AA – acetic acid, LA – lactic acid, TFA – total fermentation acid*

Efficient and effective fermentation characteristics with well preservation of nutrients are the other reason difficult to replace whole crop corn silage. However as reported by Alemayehu et al, 2019 (Table

4), the fermentation characteristics of Italian ryegrass and winter cereal mixture silages is excellent and most of the values are in the range of target values of grass and corn silage reported in Kung and shaver, (2001) and Agricultural and Horticultural Development board of the United Kingdom, AHDB Dairy, (2012). According to this report efficient fermentable characteristics of ensiled mixtures with excellent fermentation end products like high lactic acid with very low NH_3 – N, ethanol and acetic acid together with better nutrient recovery make this mixture as possible alternative silage to at least partly replace corn silage. Additionally, this result indicates that this kind of mixtures can be ensiled without additives.

Conclusions

Drought, high heat waves and ground water shortage currently altering corn crop cultivation practices in many areas of the world. Sensitivity of corn silage to climate change reveals that, management practices in relation to silage corn production governed by the current climate and local conditions must change considerably by the warming climate. It is essential to consider how crop production and feeding strategies can be adapted to this change in long-term taking into account the nutrient requirements of high producing lactating cows. Identifying alternative forage crops to fill corn silage demand prone to corn crop failure is necessary to satisfy the demand of corn silage for high producing dairy cows. When Italian ryegrass and winter cereals ensiled in mixture, it produces silage with good nutritional composition and fermentation quality compared to other grass and cereal silages ensiled alone. Efficient fermentable characteristics of ensiled mixtures with good fermentation end products like high lactic acid with very low NH_3 – N, ethanol and acetic acid together with better nutrient recovery could make this mixture a possible alternative silage to partly replace corn silage.

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Whole Genome Diversity of Indigenous Chicken Populations in Ethiopia

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Abstract

Indigenous chicken makes most of the world's poultry genetic diversity particularly in developing countries. These local breeds, which are well-adapted to extensive husbandry systems and suitable for resource-poor poultry farmers endowed with very limited means, should be thoroughly studied as a basis for enhancing their use and conservation. Understanding the link between genomic diversity and adaptability is opening the door to marker-assisted breed improvement programs. Here, we report the genomic diversity of Ethiopian indigenous chicken through discovery and characterization of 21 million SNPs (72% novel) from 27 indigenous chicken populations (n = 260 birds) using whole genome sequencing. In each population around 10 to12 million SNPs are present, of which, 40 - 47% are heterozygote. The mean SNP density for all population across all autosomes is 20 ± 5 per kb is much lower, for the Z chromosome (mean = 21) and the W chromosomes (mean = 0.4). Principal component and admixture analyses suggest the presence of four ancestral gene pools across the populations. Over 46% of the SNPs are located within genes, of which exonic and intronic SNPs account for 1.59% and 43.94%, respectively; while 31% of the exonic SNPs are non-synonymous. A large proportion of SNPs has low alternative allele frequency (AAF < 10%), although this proportion is higher in exon for potentially harmful categories like missense and stop gain/loss (> 60%) compared to neutral genes (40-45%). Genes with deleterious missense variants are included within several important biological pathways including innate immunity. This study confirms the existence of significant genomic diversity in indigenous chicken populations of Ethiopia, with most of the variants previously undescribed in commercial breeds.

***Key words**: Annotation, Chicken, Diversity, Single Nucleotide Polymorphism, Variants*

Background

Indigenous chicken makes a profound contribution to the rural economies in Ethiopia by playing a major role for the rural poor and marginalized people as a subsidiary income and consumption. Genetic diversity represents the total genetic variation among populations and several measures of diversity have been developed over years (Barrandeguy and GarcíA, 2014). Sufficient genetic variation in livestock populations is necessary both for adaptation to future changes in climate and consumer demand, and for continual genetic improvement of economically important traits (Aslam et al., 2012; Eggen, 2012; Schmid et al., 2015). To understand phenotypic variation in farm animals such as in poultry, it is essential to define all potential genomic variation within a genome (Schmid et al., 2015). Evolution of chickens and programs for their artificial selection rely on the availability of sufficient levels of genetic variation. In response to the global shift in environmental conditions and market

demands for chicken products, the diversity of village chicken is needed for future improvements programs (Muchadeyi et al., 2008).

Different techniques are involved to study genetic variations., DNA variation known as Single-Nucleotide Polymorphisms (SNPs), the most abundant sources of genome variation, have become increasingly markers of choice in genomics studies (Alderborn, 2000; Gheyas et al., 2015) with genome annotation, the link between biological or functional information and genome sequences an important gain in our understanding of how genomic variations influence phenotypes (Fulton, 2012).

Previous studies on Ethiopian chicken failed to comprehensively characterize the chicken genomic diversity in Ethiopia. Only a few Ethiopian indigenous chicken populations have been characterized using molecular markers. These are Tillili (Alemayhu, 2003; Mogesse, 2007), Jarso, Horo, Chefie, Tepi (Tadelle et al., 2003), Gellila, Debre Ellias, Melo Hamusit, Farta (Mogesse, 2007), Sheka, Konso and Mandura chicken populations. With regard to the mapping of genes of interest in indigenous Ethiopian chickens, so far a single work has been published (Wragg et al., 2012). Wragg *et al.* (2012) studied randomly selected 15 birds from 5 Ethiopian chicken populations (in Gondar, Konso, Gumuz, sheka and Guduro). Together with other breeds the study mapped phenotypic traits (e.g. skin and egg color) using genome wide association approaches. It illustrates for the first time the possibility and power to use indigenous outbreed chicken population for the fine genome mapping of Mendelian traits. Wragg and his colleagues also recommend including, a minimum of 90-110 kb SNPs for effective genome-wide associations study in village chickens.

The presence of genomic diversity in domestic chicken is of great importance and a prerequisite for rapid and accurate genetic improvement of selected breeds in various environments, as well as to facilitate rapid adaptation to potential changes in breeding goals (Nielsen, 2005). Hanotte et al., (2010), suggested that it is the high time to tap Africa's livestock genomes to better understand and exploit the genetic diversity of Africa's individual livestock breeds before they fade away. Understanding which factors shape levels of genetic diversity within genomes forms a central question in evolutionary genomics and is of importance for the possibility to infer episodes of adaptive evolution from signs of reduced diversity. There is an on-going debate on the relative role of mutation and selection in governing diversity levels (Mugal et al., 2013).

This study aims at characterizing genomic diversity through discovery of genomic variants in 27 indigenous chicken populations of Ethiopia which will be a milestone for further works on management and conservation of chicken genetic resources, studying the genetic mechanism under lying local chicken adaptation.

Materials and Methods

Sampling Strategy and Blood sample collection

Blood samples were collected from 27 chicken populations in Ethiopia (**Error! Reference source not found.**). Samples included 103 cocks and 157 hens. Except the improved Horro, Meseret, Tsion Teguaz, Jarso and local Horro populations, 10 chickens from each village were sampled. One or two chicken were sampled per household. Improved Horro was sampled from a breeding stock of 8th generation under selection at Debre Zeit Agricultural Research Center and used as a reference population. Unlike other populations, Jarso and local Horro sequences were obtained and included from the previous

studies. Photographs and weight of each bird were taken. The average weight of sampled chicken was 1.26 Kg with age ranges of 5 to 36 months. Sampling considered different agro-ecological zones, altitudes ranging from 729-3500 meters), marketing points, and chicken phenotypic characteristics. From the wing vein of each chicken, 50 - 250 µl of whole blood were drawn with syringes using cryo-tubes filled with 1.5 ml absolute ethanol (100 %) following the guidelines available at https://www.sheffield.ac.uk/nbaf-s/protocols_list.

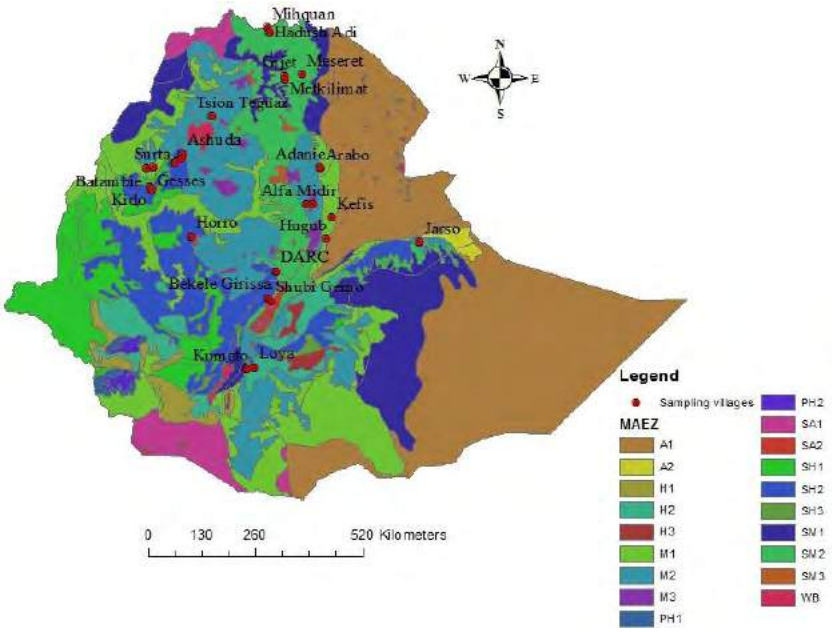


Figure 1. Agro-ecological map of Ethiopia with sampling site (MOA, 2000).

WB = Water body; A1 = Tepid to cool arid mid highlands; Hot to warm semi-arid lowlands; H1= Hot to warm humid lowlands; H2 = Tepid to cool humid mid highlands; H3 = Cold to very cold humid sub afro-alpine to afroalpine; M1 = Hot to warm moist lowlands; m2 = Tepid to cool moist mid highlands; M3 = Cold to very cold moist sub-afro alpine to afroalpine; SA1= Hot to warm semi-arid lowlands; SA2 = Tepid to cool semi-arid mid highlands; Sh1= Hot to warm sub-humid lowlands; SH2 = Tepid to cool sub-humid mid highlands; SH3 = Cold to very cold sub-humid sub-afroalpine to afro-alpine; SM1= Hot to warm sub-moist lowlands; SM2 = Tepid to cool sub-moist mid-highlands; SM3 = Cold to very cold sub moist sub afro alpine to afroalpine; PH1 = Hot to warm per humid lowlands; PH2=Tepid to cool per-humid mid highlands.

DNA isolation

Total DNA was extracted from chicken whole blood at the BecA-ILRI Hub, Nairobi, Kenya facility (http://hub.africabiosciences.org/) using the Qiagen DN easy blood and tissue kit protocol (Lwelamira *et al*., 2008). To evaluate the DNA concentration a Thermo Scientific NanoDrop spectrophotometer 2000c was used. The integrity of DNA was confirmed by agarose gel electrophoresis whereby 20 ng/µl genomic DNA samples were loaded with 1 µl loading dye (6X) on 1 % agarose gel containing 2.5 µl gel red at a voltage of 7/cm for 60 minutes, 3 µl of lambda DNA of size of 48,500 bp and at concentration of 20 ng/µl) was used as size marker and the gel was then examined using UV light by comparison using GelDoc-It^2 Imager to check the extracted DNA quality and quantity. The genomic DNA from (N = 284) was normalized to a final volume of 100 µl and final concentration of 50 ng/µl and sent to Edinburgh Genomics, UK, for whole genome sequencing.

DNA quality checking (QC) and library Preparation

The library prep, QC and sequencing were performed at the Edinburgh Genomics facility. Genomic DNA (gDNA) samples were evaluated for quantity and quality using an AATI Fragment Analyzer and the DNF-487 Standard Sensitivity Genomic DNA Analysis Kit. The AATI ProSize 2.0 software was used to provide a quantification value and a quality (integrity) score for each individual gDNA sample. Genomic DNA samples having a quality score of > 7 and with high molecular weight were used. Based on the quantification results, gDNA samples were normalized to the concentration and volume required for the Illumina SeqLab TruSeq Nano library preparation method using the Hamilton MicroLab STAR. Next Generation sequencing libraries were prepared using Illumina SeqLab specific TruSeq Nano High Throughput library preparation kits in conjunction with the Hamilton MicroLab STAR and Clarity LIMS X Edition. The normalized gDNA samples were then sheared to a 450 bp mean insert size using a Covaris LE220 focused-ultrasonicator. The inserts were ligated with blunt ended, Atailed, size selected, TruSeq adapters and enriched using 8 cycles of PCR amplification.

Library QC and sequencing

The libraries were evaluated for mean peak size and quantity using the Caliper GX Touch with a HT DNA 1k/12K/HI SENS LabChip and HT DNA HI SENS Reagent Kit. Those libraries were then normalized to 5nM using the GX data and the actual concentration was established using a Roche LightCycler 480 and a Kapa Illumina Library Quantification kit and Standards. The normalized libraries were denatured and pooled in eights for clustering and sequencing using a Hamilton MicroLab STAR with Genologics Clarity LIMS X Edition. Libraries were clustered onto HiSeqX Flow cell v2.5 on cBot2s and the clustered flow cell is transferred to a HiSeqX for sequencing using a HiSeqX Ten Reagent kit v2.5. The samples were sequenced at a genome coverage of ~5-90 X (mean=36.1 x). Demultiplexing is performed using bcl2fastq (2.17.1.14), allowing 1 mismatch when assigning reads to barcodes.

Adapters (Read1: AGATCGGAAGAGCACACGTCTGAACTCCAGTCA,
Read2: AGATCGGAAGAGCGTCGTGTAGGGAAAGAGTGT)
are trimmed during the demultiplexing process.

Mapping and variant calling

The pipelines for mapping and variant calling included: mapping reads against reference genome using BWA-mem, sorting BAM file, removing duplicated reads with PICARD, Base Quality Score Recalibration (BQSR) with GATK, calling variants using GATK, Variant Quality Score Recalibration (VQSR) for variant filtration with GATK, and finally selection of only bi-allelic SNPs which passed the VQSR step.

Mapping with BWA-mem: High quality paired end reads (FASTAQ format) were aligned to the chicken (Gallus gallus) reference genome sequence (Gallus_gallus-5.0 or galGal5) (https://www.ncbi.nlm.nih.gov/genome/?term=Galus+galus+5), using Burrows-Wheeler Aligner software package (http://sourceforge.net/projects/bio-bwa/files/) with the command 'mem -t 8 -k 32 –M -R' (where –t = no. of threads; -k = min seed length; -M= Mark shorter split hits as secondary (for Picard compatibility) which permits high-quality queries for longer sequences as it is fast and accurate (Li and Durbin, 2010); and –R for defining read groups.

The alignment output generated were stored in the SAM format and then converted to BAM formats using PICARD tools. Duplicated reads originating from a single fragment of DNA during sample preparation (such as library construction using PCR) were marked and removed using PICARD's Mark Duplicates command (https://broadinstitute.github.io/picard/command-line-overview.html#MarkDuplicates).

Base quality score recalibration (BQSR) and Variant calling: - BQSR is a data pre-processing step that detects systematic errors made by sequencers in estimating the quality score of each base call. Base quality score is an important parameter for variant calling as it expresses confidence that the base has been called correctly. Unfortunately, the scores produced by the machines are subject to various sources of systematic technical errors, leading to over- or under-estimated scores. The BQSR step applies a machine learning algorithm to model these errors empirically and adjust the quality scores accordingly by considering a number of covariates such as sequencing context of the base, position in read or sequencing cycle (https://gatkforums.broadinstitute.org/gatk/discussion/44/base-quality-score-recalibration-bqsr). Variant calling from each sample was performed in gVCF mode for cohort analysis using GATK's Haplotype Caller. Joint genotyping of samples from each population were done using GATK's GenotypeGVCF tool for downstream analysis. Variant Quality Score Recalibration (VQSR) was also performed to increase sensitivity (identifying the real variants) and specificity (identifying false positives) using GATK followed by selection of only bi-allelic SNPs that passed the VQSR step. For the VQSR step we used 1M validated SNPs and 15 SNPs from dbSNP for recalibration purpose.

Population structure and genome wide nucleotide diversity

Population structure and relationships between samples were established using Principal Component Analysis (PCA) using smartpca program in eigenstrat version 6.0 (Patterson et al., 2006; Price et al., 2006) PCA was performed using all SNPs from 27 populations (n = 21,303,759). The top three principal components (PCs) provided the clearest separation of the data and were used to construct the PCA plot. Apart from the PCA, the genetic structure of each population was also assessed unsupervised, using ADMIXTURE version 1.3.0 (Alexander et al., 2009). Global admixture analysis were ran for K = 2 to K = 10 assumed ancestors from 651,417 LD pruned SNPs. The optimal K value was determined based on the lowest cross validation error. The average genome nucleotide diversity (π) for each population was determined using VCFtools version 0.1.13 in 20 kb windows over a 10 kb sliding step (Danecek et al., 2011).

Functional annotation and enrichment analysis of Non synonymous genes

To predict their functional consequence, SNPs were annotated against Ensemble chicken gene database (release 92) using the software package ANNOVAR (Wang et al., 2010). The effects of non-synonymous SNPs on protein function were predicted based on evolutionary conservation using the Sorting Intolerant from Tolerant (SIFT) prediction algorithm which depends on the degree of conservation at individual amino acid (AA) positions (Sim et al., 2012). Using multiple alignment of homologous but distantly related peptide sequences, SIFT calculates normalized probabilities (SIFT score) of observing all possible AA residues at a position (Gheyas et al., 2015; Ng, 2003). If the SIFT score is greater or equal to 0.05 the variant is considered evolutionary tolerant (TOL), whereas variants with score less than 0.05 are regarded as intolerant (INTOL) and potentially deleterious (Choi and Chan, 2015; Kumar et al., 2009; Sim et al., 2012). The SNPs were further checked for their overlap with 1.1 million conserved elements (CE) obtained from Roslin institute (Eory, L. *et al*., unpublished data; personal communication). These CEs were generated from multiple alignment of sequence data from 48 bird species and 1 anole lizard species using Genomic Evolutionary Rate Profiling (gerp++) (Davydov et al., 2010).

The proportion of homozygous SNPs were calculated using the "stat" option of the VCFtools version 0.1.113 (Danecek et al., 2011). To establish the biological significance of a list of genes carrying potential functional SNPs, the DAVID Bioinformatics Resources 6.8 (DAVID; Huang et al., 2009a, 2009b) was used. This allowed performing enrichment analysis of the Gene Ontology (GO) and the

Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathways (KOBAS version 3.0, http://kobas.cbi.pku.edu.cn/). For both analyses the Fisher exact P value < 0.05 default threshold were considered

Results

Sequencing and variant calling

The average number of paired sequence reads generated from individual samples in each population ranged between about 202 million (Hadush Adi) and 475 million (Surta) (Table 1), resulting in average genome coverage of about 22X to 44X. On average about 88% of the bases were covered by at least 5 reads and > 90% covered with at least 10 reads (**Table 1**). More than 98% of read pairs in all samples were mapped to the Galgal 5.0 reference genome. The mean sequence depth of the entire chicken population sampled is about 39X.

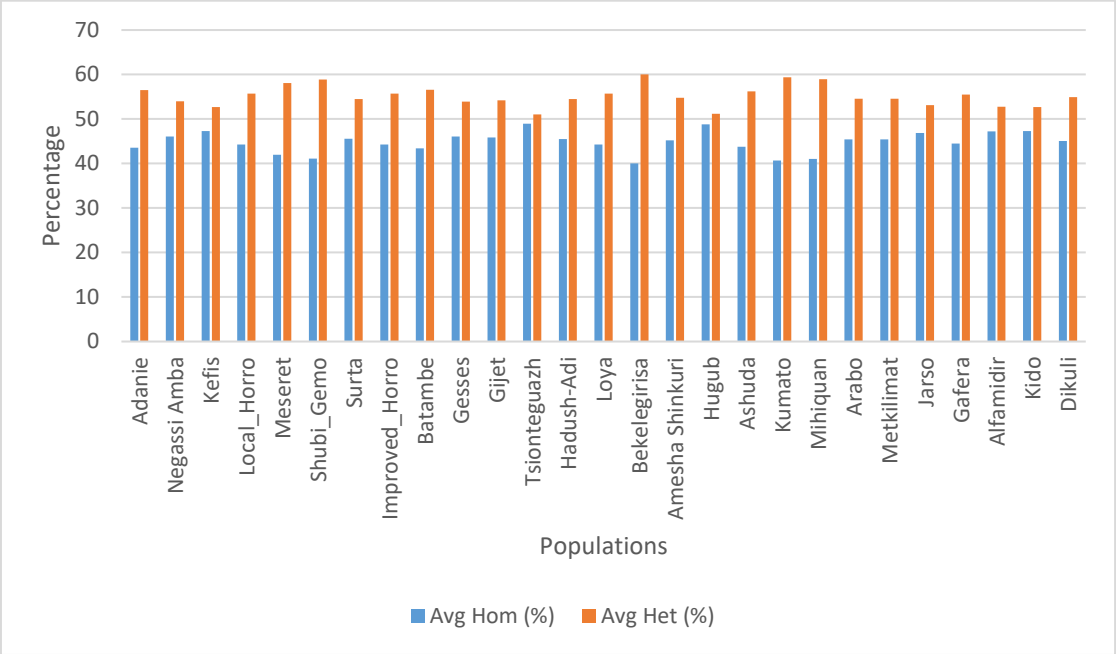
Variant calling and filtration resulted in the detection of about 21M SNPs (n=20,867,451) in total from 27 population combined. The number of SNPs detected from individual population ranges from 10 to 12 million. The mean SNP density reported in this study are 21 SNPS per kb (± 5) or 1 SNP per every 48 bases. From the total SNPs, about 28.12 % (5,868,599) of the SNPs are already reported in dbSNP (build 147) which currently contains ~21 million SNPs (n = 21,303,759) for chicken; while the rest of the SNPs (n = 14,998,852) are novel. Much lower SNP density is found in sex chromosomes than autosomes (
Figure **4**). The genome landscape plot of genes in the entire and individual chromosomes is 1 Kb genome coverage is given in

Figure ***3***. The white ridges as evidenced by samtools tview with no density coverage of SNPs are the gaps because of mapping issues due to gaps in the reference genome where by no mapping was possible for GC repeat regions. Wide spread variations have been noted in SNP density across chromosomes. The lowest SNP density was reported for Chromosome W and the highest SNP density was reported for Chromosome 26 followed by chromosome 6. The peak number of Non-synonymous SNPs where observed in chromosome 16. Except for Jarso about 11 to 15 % of novel SNPs are discovered from each chicken population (Table 2). The peak private allele is owned by Jarso chicken (n=280448) and the lowest for Batambe chicken (n = 34532). Alternate allele frequency of the chicken population considered ranges from 0.35 for loya and 0.39 for local Horro and Hugub chicken populations. About 18 to 19 % of the SNPs overlapped the conserved elements. The population with a relatively highest putatively functional variant is Kumato followed by local Horro chicken population). The proportion of SNPS that were found heterozygous 55.21 % for the overall chicken population (n = 284). Bekele Girrisa chicken populations have the highest average heterozygous SNPs (60%) followed by Kumato chicken population (59%), n = 10). The mean genome nucleotide diversity (π) of the entire chicken population is 0.02 $\pm$0.001. The lowest average hetrozygous SNPs is reported for Hugub chicken populations (about 40%). The average transition to tranversion ratio of detected SNPs is 2.35. The highest number of nucleotide substitution was recorded for C >T and G > A (Figure 5).

Table 1. Summary results on sequencing and mapping of reads

Sample	Average number	MR	MD (X)	5_b	10_b	20_b	40_b (%)
Batambie	436294418	99.06	40.33	87.04	78.69	73.73	60.20
Surta	475115042	98.72	41.51				
Amesha	427316335	99.21	39.02				
Hugub	360343162	99.48	35.67	90.11	88.02	81.85	40.17
Kefis	461097537	99.41	44.42	90.41	88.35	82.11	43.33
Gafera			43.45				
Tsion	244270731	99.29	22.04	75.64	64.63	47.75	9.31
Adanie	376903935	99.27	35.89	90.03	87.96	81.94	40.23
Arabo	309414324	99.02	31.30	89.54	85.79	72.33	24.10
Alfamidir	342600741	97.29	89.39	83.64	74.18	34.84	
Negasiamba	362919193	98.96	35.52				
Ashuda	436640467	99.07	39.76				
Dikuli	418411806	99.16	36.93				
Gesses	373183155	99.44	35.66	90.16	88.14	82.14	40.35
Kido	357193268	82.92	37.30				
Improved Horro	351003978	99.46	32.51				
Meseret	433954119	99.30	39.24				
Bekelegirisa	383898518	98.95	36.38				
Shubigemo	421492594	99.27	39.09				
Jarso	368646704	99.75	25.96	88.76	85.68	74.00	7.86
Local Horro	387105936	99.74	28.68	88.78	86.10	76.57	9.90
Kumato	404282348	99.45	38.13				
Loya	461026931	99.41	42.61				
Hadush Adi	201579979	98.29	39.68	87.47	79.22	57.72	41.98
Mihiquan	335214296	99.45	34.37				
Gijet	439628764	99.40	43.08	90.23	88.28	82.73	45.19
Metkilimat	339764180	99.36	33.99				

MR: Total number of reads mapped to the Galgal 5.0 reference genome; MD: The mean sequence depth or the genome sequence coverage; 5_b: Percentage of the genome with bases covered by at least 5 reads; 10_b: Percentage of the genome with bases covered by at least 10 reads; 20_b: Percentage of the genome with bases covered by at least 20 reads; 40_b: Percentage of the genome with bases covered by at least 40 reads



Home=Average Homozygosity; Avg Het=Average Heterozygosity

Figure 2. Average percentage of homozygous and heterozygous SNPs (%) in Ethiopian indigenous chicken populations.

Table 2. Variant statistics within chicken populations from Ethiopia

Population	N	nSNPs	Known (%)*	Novel (%)	PA	µAAF	TS/TV	PI	CE (%)	PFV (%) **
Batambie	8	11278325	88	12	34532	0.384574	2.44	0.003646	2117632 (18.78)	8176 (0.07)
Surta	9	11769723	87	13	40052	0.371836	2.44	0.003757	2209322 (18.77)	8791(0.07)
Amesha Shinkuri	10	12037555	86	14	45467	0.366294	2.43	0.003718	2258138 (18.76)	9100(0.08)
Hugub	10	10873408	90	10	75551	0.390987	2.44	0.003442	2037719 (18.74)	7843(0.07)
Kefis	10	11606161	87	13	76463	0.374837	2.43	0.003609	2172148 (18.72)	8623(0.07)
Gafera	10	11938205	86	14	47192	0.36822	2.44	0.003663	2237236 (18.74)	9051(0.08)
Tsion	10	11646405	87	13	91269	0.377861	2.43	0.003488	2181362 (18.73)	8644(0.08)
Adanie	10	12254914	85	15	166779	0.363589	2.43	0.003662	2293708 (18.72)	9130(0.07)
Arabo	10	12025464	86	14	116991	0.36956	2.43	0.003491	2249348 (17.70)	9181(0.08)
Alfamidir	10	11302647	87	13	79002	0.382912	2.43	0.00326	2119442 (18.75)	8445(0.07)
Negasiamba	10	11366554	87	13	71076	0.381866	2.43	0.00336	2128289 (18.72)	8385(0.07)
Ashuda	10	11430789	87	13	49469	0.377795	2.43	0.003541	2140547 (18.73)	8637(0.08)
Dikuli	10	12009063	86	14	52605	0.360253	2.43	0.003695	2254716 (18.78)	9105(0.08)
Gesses	9	11313583	87	13	47730	0.383376	2.44	0.003444	2121533 (18.75)	8411(0.07)
Kido	9	11369167	86	14	146190	0.384662	2.42	0.003504	2116126 (18.61)	8502(0.07)
Improved Horro	30	11154784	88	12	48121	0.386675	2.44	0.003436	2092166 (18.76)	8043(0.07)
Meseret	10	12239534	86	14	121862	0.358991	2.43	0.003801	2292807 (18.73)	9405(0.08)
Bekelegirisa	10	12449396	86	14	96851	0.356569	2.43	0.003866	2334214 (18.75)	9637(0.08)
Shubigemo	10	11982320	87	13	91797	0.361542	2.44	0.003797	2253098 (18.80)	9027(0.08)
Jarso	14	12218592	100	0	280448	0.354585	2.45	0.003338	2296595 (18.80)	9442(0.08)
Local Horro	6	10602823	89	11	58140	0.392005	2.46	0.003577	1990141 (18.77)	7470(0.07)
Kumato	10	12595174	85	15	83208	0.353946	2.43	0.003949	2362678(18.76)	9843(0.08)
Loya	10	12300789	85	15	113533	0.351472	2.43	0.003882	2304034(18.73)	9593(0.08)
Hadush Adi	9	11542439	87	13	99497	0.375424	2.44	0.003741	2169976 (18.80)	8508(0.07)
Mihiquan	10	12442251	85	15	161855	0.354912	2.44	0.003865	2333135 (18.75)	9622(0.08)
Gijet	9	11751551	87	13	76662	0.368866	2.45	0.003745	2208579 (18.79)	8837(0.08)
Metkilimat	10	12203356	86	14	97841	0.361624	2.43	0.003782	2289221 (18.76)	9411(0.08)

N=Number of samples analyzed; **nSNPs** = total Number of SNPs obtained; *Check already reported snps in dbsnp; PI=Nucleotide diversity;**pfv= putatively functional variants (Del, stopgain/loss, splicing and High Impact); PA= Private SNPs for each population; µAAF-Average Alternate allele frequency; %PFV is based on the total SNPs detected from each population.

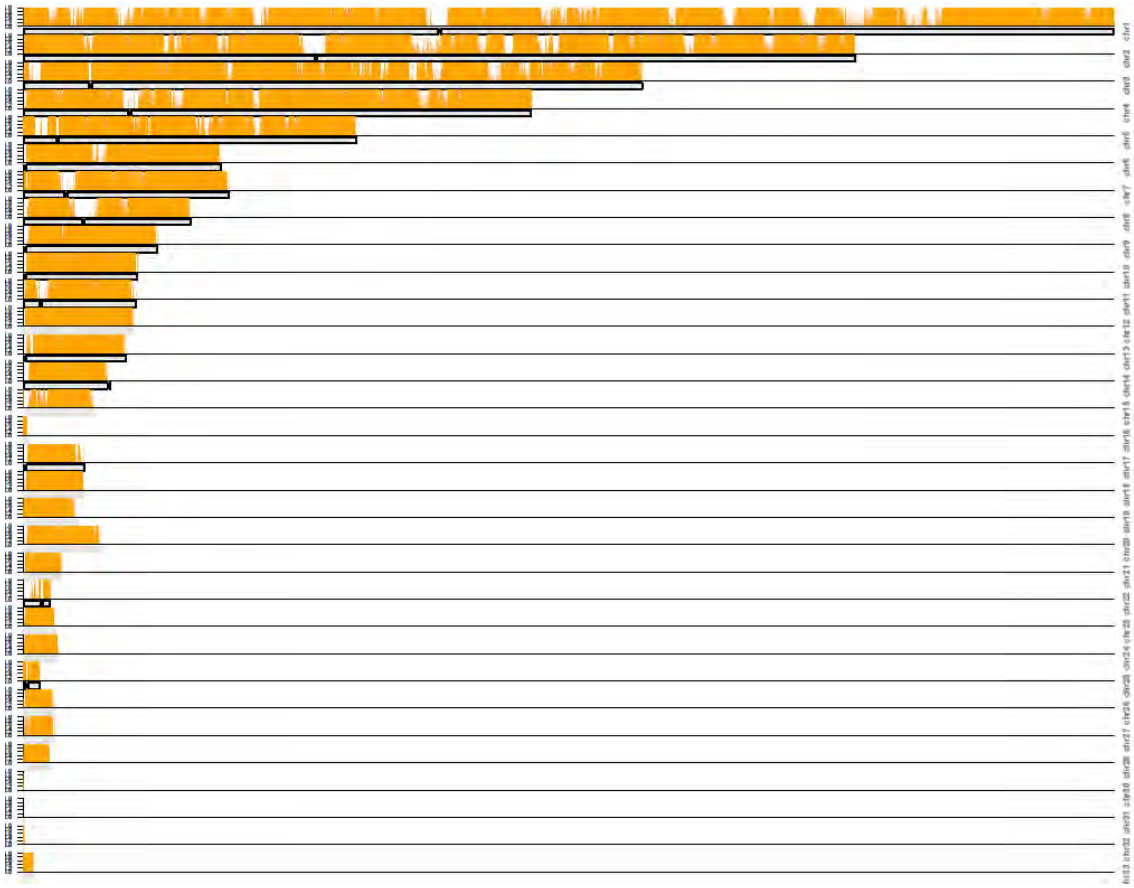
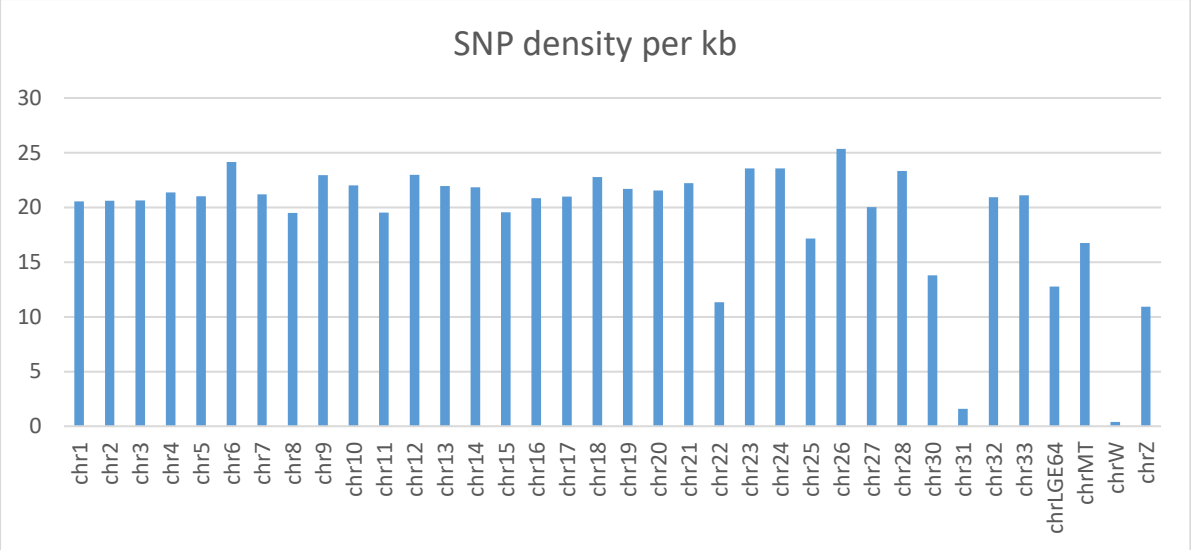


Figure 3. Chromosome-wise SNP distribution plot across the Ethiopian indigenous chicken genome. The x-axis denotes the chromosome size (1 Kb), and the y-axis indicates the chromosomes. If no SNPs are found in a block, we used a white color. Therefore, the deeper the color, the higher the number of SNPs. The window size used to count the SNPs was one KB.



Chromosomes	Mean ± SD SNP density
Macro(Chr1-5)	21±4
Intermediate (Chr 6–10)	22±4
Micro (Chr 11–28, Chr30-32, Chr MT, Chr LGE64)	19±6
(Chr W)	0.4
Chr Z	11
All autosomes	20±5

Figure 4. Mean SNPs density across 1 Kb chicken chromosomes based on ~21million SNPs.

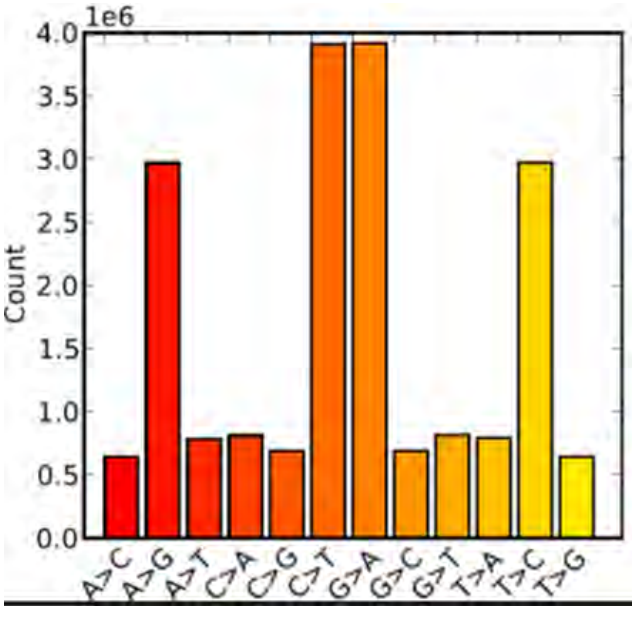


Figure 5. Rate of nucleotide substitution across Ethiopian indigenous chicken population genome

Population structure and genomic diversity

PCA plots show presence of 6 potential clusters of populations, with PC1 and PC2 jointly explaining 41.4% of the total genetic variance (**Error! Reference source not found.**). PC1 separated improved Horro from the rest of non-improved chicken populations. Jarso and Hugub populations were separated from improved horro and other populations by PC2. The rest of the populations were placed quite close in the PCA plot, although they could be separated into three more clusters. Admixture analysis suggested 4 gene pool groupings from 651,417 LD pruned SNPs as the lowest cross-validation error was observed for K= 4 (Figure *7*). LD pruned principal component analysis (left) and optimum admixture ($2 \leq K \leq 4$ plot (right) for Ethiopian indigenous chicken population is indicated in **Error! Reference source not found.**. Admixture analysis after pruning also clustered Improved Horro, Jarso, hugub populations in gene pool one with a certain level of admixture from gene pool two (*Figure 8*). Both PC and Admixture plots illustrate clustering of Improved Horro, Arabo, Jarso, and Hugub populations independently, leaving the rest of the populations altogether. In other words, the majority of the population shares quite a lot of SNPs and are closely clustered. PCA analysis plots prior to LD pruning are also presented in *Figure 6*.

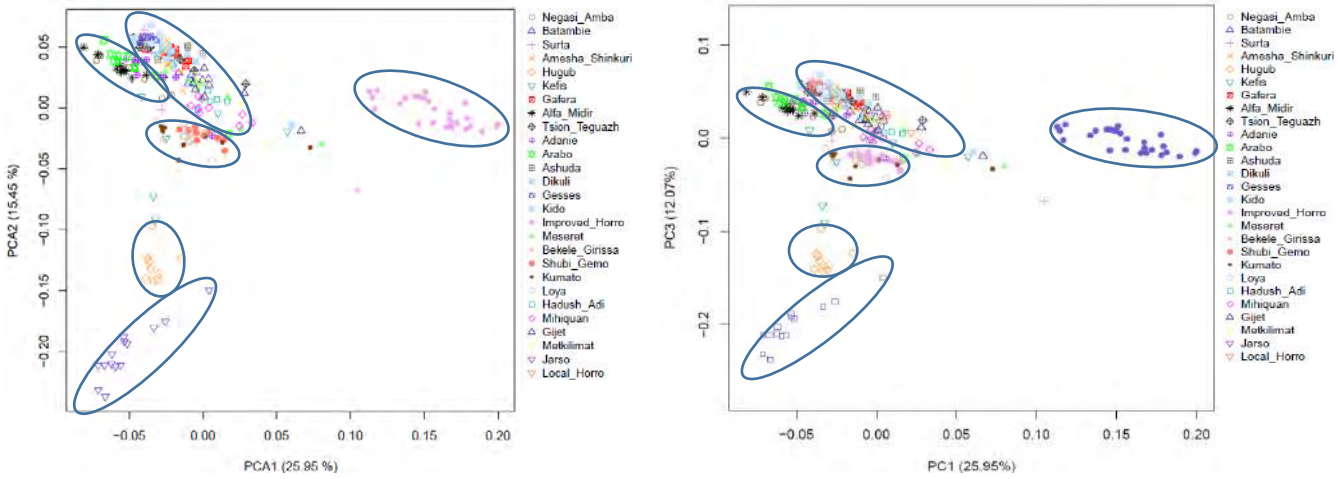


Figure 6. Principal component analysis plot of non-filtered (20,867,451) (left) and LD pruned SNPs (right).

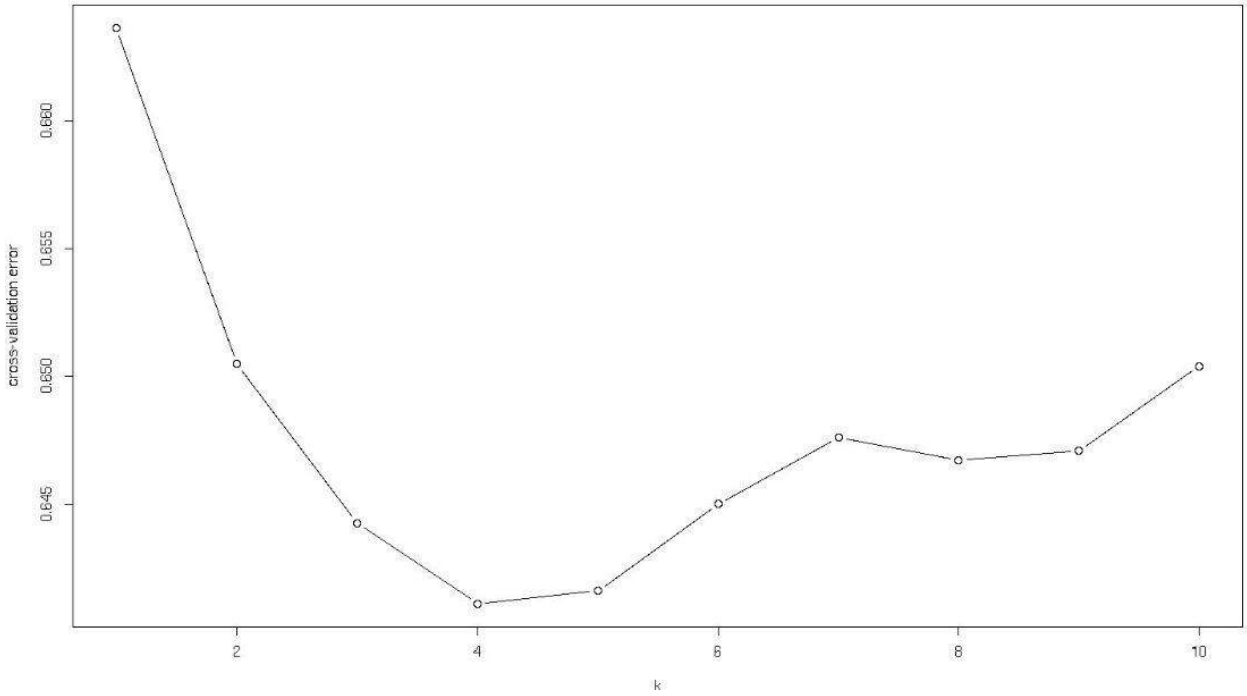


Figure 7. Cross validation errors of different k values used for admixture analysis

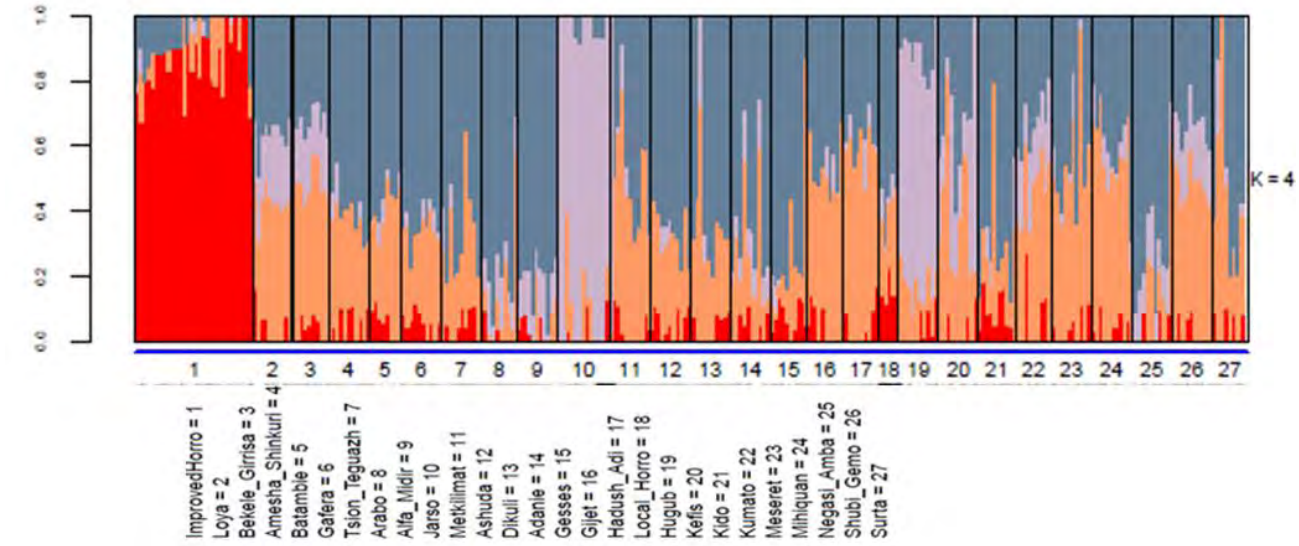


Figure 8. The admixture plots for Ethiopian indigenous chicken populations (K = 4).

Coding and non-coding variants

All chicken with ≥1132563 novel variants are reported except Jarso chicken population with zero novel variants. About 2 million or more conserved elements are reported except for Kumato population without these elements. About 7800 deleterious and high impact SNPs have been reported for indigenous chicken populations except Kumato. Annotation of 21M SNPs against ENSEMBL gene annotation database shows that 46.36 % of SNPs are located within genes (intronic + exonic + UTRs + splicing) and the rest are available outside genes (intergenic and up/downstream). However, only 1.6 % (n = 331,968) of the SNPs are in protein-coding regions (i.e. exonic) (Table 4). SNPs in exonic regions were further classified into synonymous (variants which do not alter amino-acid sequence in proteins), non-synonymous (variants that changes amino-acid sequence in protein), and stop gain or loss (variants that leads to gain or loss of stop codon) types. The synonymous and non-synonymous (AA-altering) number of SNPs are 190,041(0.48 %) and 100293 (0.98%), respectively. Whereas, the other AA altering variant, number of stop gain/loss accounts for about 0.36% (n = 1,209). Even though non-synonymous SNPs change amino acid sequence within a protein, the effects are not always harmful or radical on protein function. Using SIFT, 21.9 % of the non-synonymous variants (n = 44, 553) were predicted as 'intolerant' (INTOL) having radical effect, 64.94% (135,917) were predicted 'tolerant' (TOL), whereas the prediction for other variants had low confidence level (Figure 11). Much higher SNP density was observed in Chr16 (probably because it contains highly variable MHC regions) and also in smaller chromosomes (chr25-33). Smaller chromosome may be gene rich and hence you see greater density (Figure 10).

Apart from the amino-acid altering variants, other potentially functional categories are also reported in this study, such as splicing variants (0.006%); variants in 3′and 5′ UTRs with possible roles of regulating protein translation (0.82 %); those within 1 kb up- or downstream of transcription start or end sites (3.06 %) with possible roles on transcriptional regulation; and finally, the SNPs belonging to ncRNAs (2.61%) (Table 5).

Table 4. Summary of annotation of SNPs and their alternative allele frequency (AAF) in Ethiopian chicken populations

Annotation category	Number (%)	Mean AAF(SD)	No. detected from >10 populations with mean AAF > 0.9 (%)	No. detected from > 27 populations with mean AAF > 0.9 (%)	No. of private SNPs with AAF > 0.9 (%)
Intergenic	10,556,684(50.59)	0.26 (0.26)	435444(2.087)	282933(0.014)	9883(0.0005)
Intronic	9,169,275(43.94)	0.26 (0.26)	366476(1.76)	234965(0.011)	5377(0.0003)
Upstream/downstream	638532(3.06)	0.25 (0.26)	24616(0.12)	14257(0.0007)	505(0.00002)
Exonic	331968(1.59)	0.24 (0.26)	14954(0.07)	9606(0.0005)	482(0.00002)
-Nonsynonymous	100293(0.48)	0.21(0.25)	4015(0.02)	2580(0.0001)	154(0.000007)
-Nonsynonymous deleterious	24728(0.11)	0.14 (0.18)	329(0.002)	152(0.000007)	25(0.000001)
-Nonsyonymous tolerated	75565(0.36)	0.23 (0.27)	2428(0.012)	2428(0.0001)	129(0.000006)
-Stop-gain/loss	1269 (0.006)	0.18 (0.22)	22(0.0001)	11(0.0000005	1(0.00000005)
Synonymous	190041(0.91)	0.26 (0.27)	9417(0.045)	6055(0.0003)	278(0.00001)
UTR3'/UTR5'	171,175(0.82	0.24 (0.25)	6550(0.031)	4067(0.0002)	89(0.000004)
ncRNA	544429 (2.61)	0.25 (0.25)	19187(0.092)	12357(0.0003)	584(0.00003)
Splicing	1269(0.006)	0.24 (0.26)	48(0.0002)	22(0.000001)	2(0.0000001)
Conserved elements	3859925(18.5)	0.26(0.00)	158053(0.76)	102316(0.005)	2252(0.00011)

Upstream: a variant that is located in the 1-kb region upstream of the gene start site; stop gain: a non-synonymous (ns) SNP that leads to the creation of a stop codon at the variant site; stop loss: a non-synonymous SNP that leads to the elimination of a stop codon at the variant site; splicing: a variant within 2 bp of a splice junction; downstream: a variant that is located in the 1-kb region downstream of the gene end site.

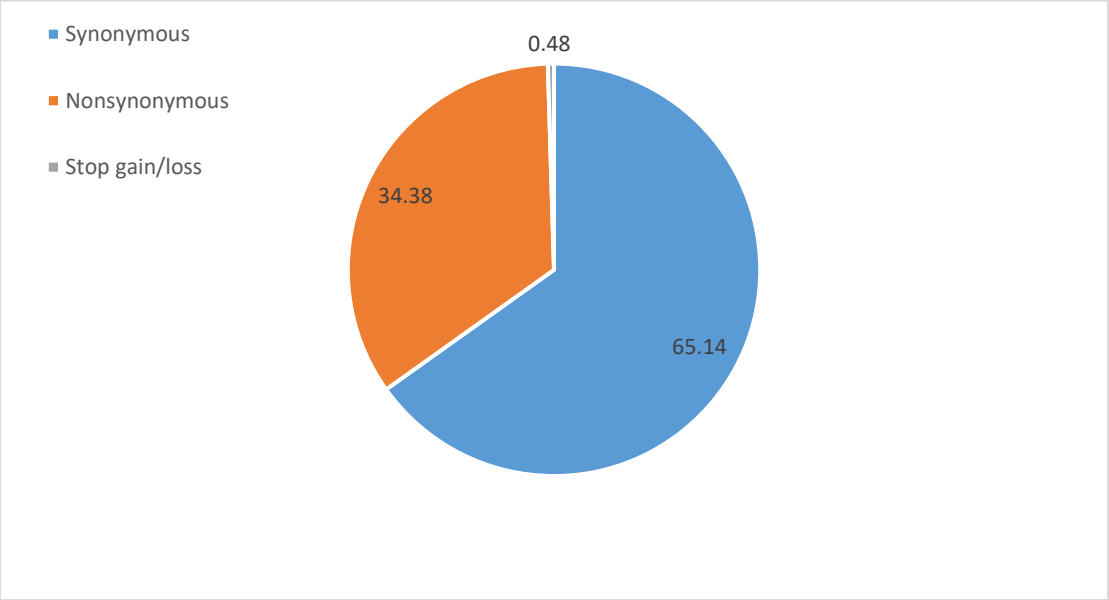


Figure 9. Exonic variant summary in each annotation category based on ANOVAR (%)

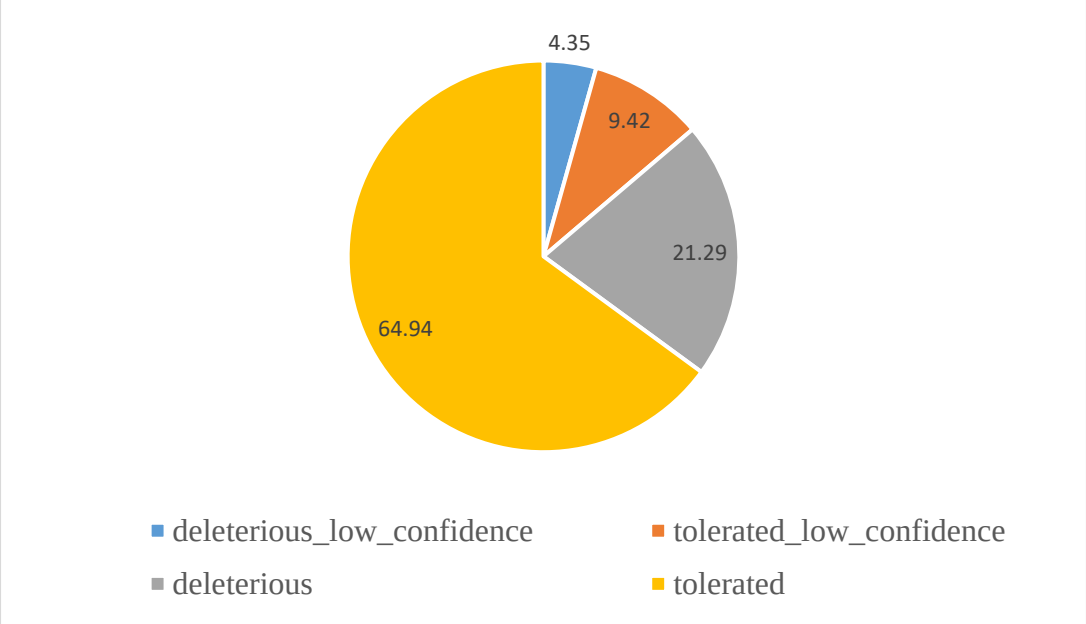


Figure 10. VEP based SIFT analysis for Amino acid altering (non-synonymous and stop gain/loss) SNPs (%).

SNPs within evolutionary conserved elements

The 21 million SNPs were annotated against 1.1 million conserved elements (CEs) across 48 birds plus a lizard sequences (Eory, L. *et al*., unpublished data; personal communication). These CEs covers about 2.1% of chicken genome (total length of CEs is 186,488,363 bases). The total number of SNPs that overlapped with CEs is 3,859,925 (18% of the 21M SNPs), of which 2,619,665 are reported in dbsnp, while the rest are novel.

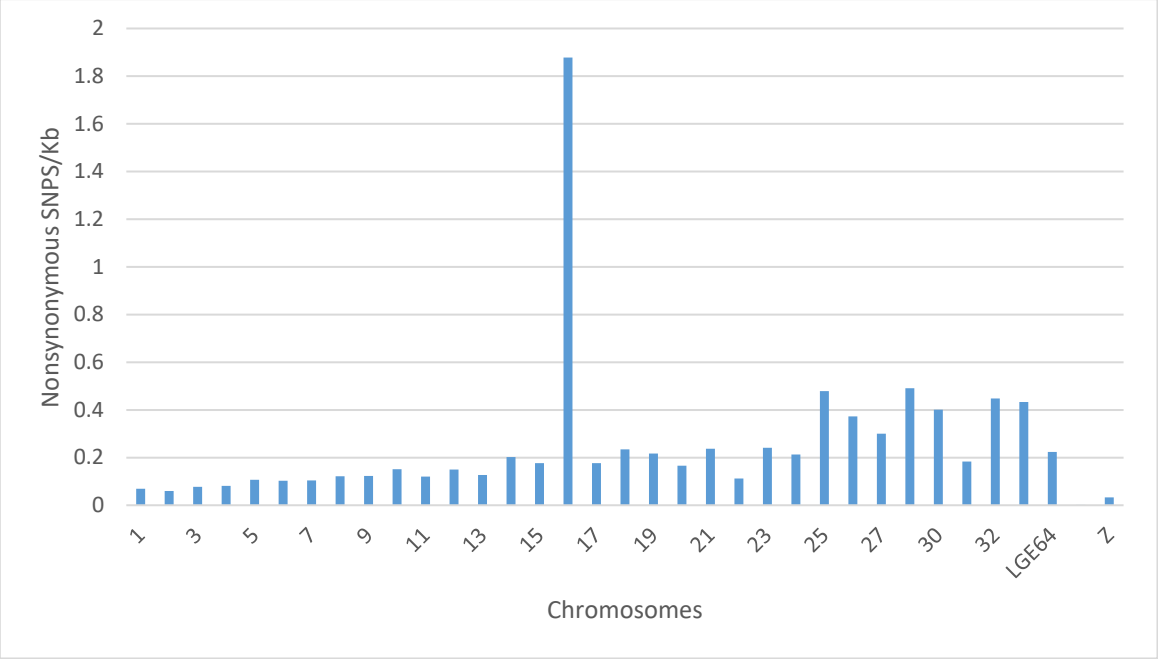


Figure 11. Non synonymous number of SNPs/Kb across chromosomes

Allele frequency spectrum of SNPs in different annotation categories

Frequency spectrum of non-reference or alternative alleles (AAF) of variants from different annotation categories were compared (Figure 13-14). The allele frequency distribution of different annotation categories showed that the largest proportion of variants fell within the AAF bin of ≤ 10%. However, the proportion was higher for potentially harmful variants like deleterious missense and stopgain/loss (> 60%) compared to neutral categories like intergenic, intronic, and synonymous (< 40%). This is expected as potentially detrimental SNPs are expected to be mostly low frequency. However, contrary to our expectation, we did not find any variation in the AAF pattern of of SNPs within CE category with potentially neutral variants. SNPs that are potentially function or deleterious but are present in high frequency (e.g. AAF > 0.9) is expected to have greater impact and may be under selection. Table 4 shows the number and percentage of putatively functional SNPs that are present in high frequency and were detected from 27 populations.

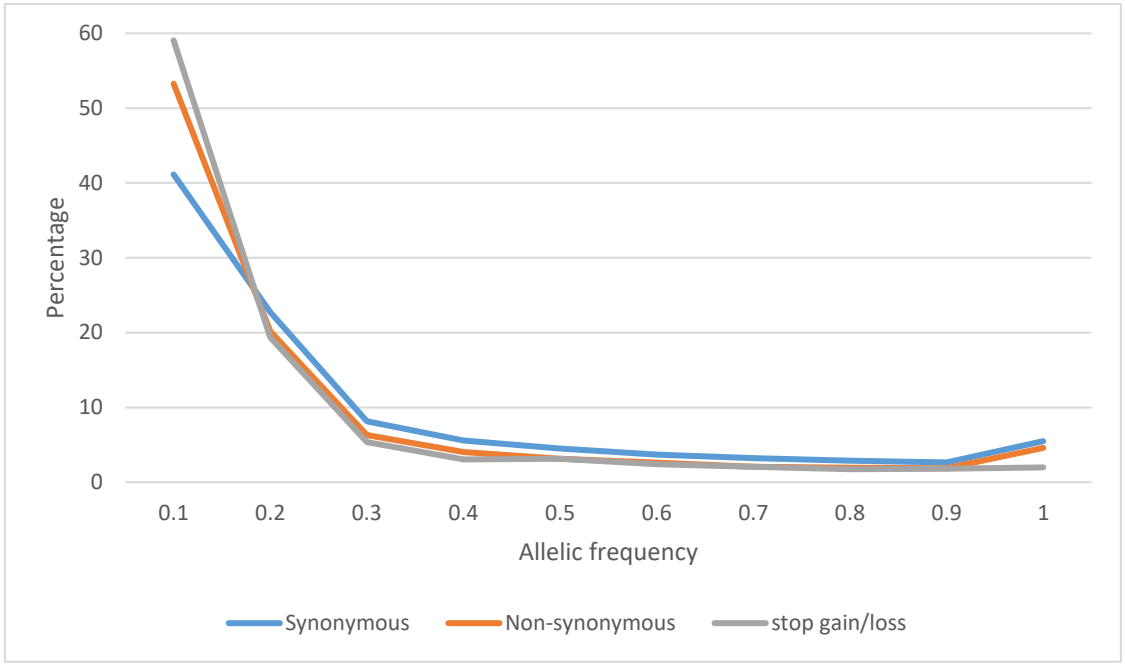


Figure 12. AAF spectrum of synonymous, nonsynonymous and stop gain/loss SNPs

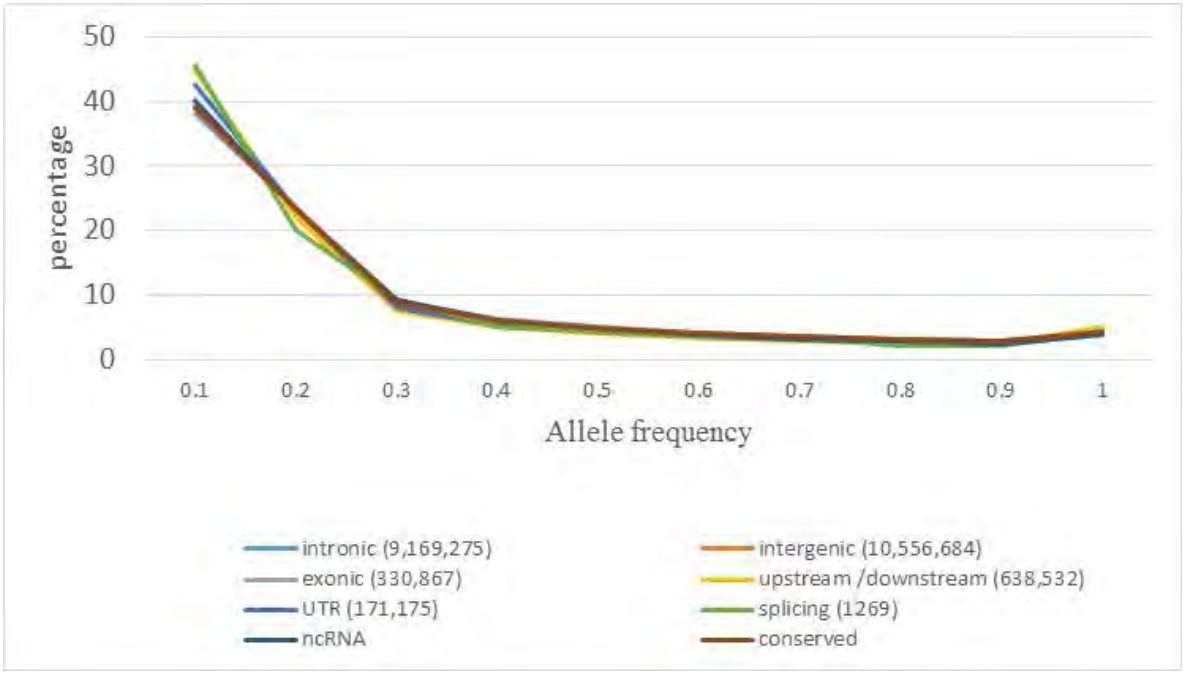


Figure 13. AAF spectrum different variants (left); AAF spectrum of synonymous, nonsynonymous and stop gain/loss SNPs.

Functional annotation and enrichment Analyses

Seven hundred ninety five reported functionally annotated found from the entire 27 population were extracted and checked for their functions and biological pathways with highest stringency. Go term enrichment analysis shows significant ($P \leq 0.05$) GO terms related to innate antibacterial and antifungal immunity response (IPR000157; Toll/interleukin-1 receptor homology (TIR) domain, IPR007110); Immunoglobulin-like fold and energy biosynthetic ((GO: 0016887; ATPase) activity, (GO: 0006183; GTP biosynthetic process), (GO: 0006228; UTP biosynthetic process), GO: 0006241; CTP biosynthetic process)) (Table 2). In a similar fashion 385 nonsynonymous deleterious genes (352 reported) detected in 10 populations where functionally annotated and Go term enrichment analysis gave genes responsible mainly for DNA repair and binding(Go:0042162, Go:0006281), ATP binding (GO:0005524) and WD40 repeat domains(IPR017986, IPR015943 , IPR001680) . Annotation of non-synonymous deleterious SNPs with AAF > 0.9 and their functional characterization has confirmed genes attributed to Methyltransferase, Protein authophosphorylation, a class of nuclear body called promyelocytic leukemia (PML).

Table 3. Go terms enriched for non-synonymous deleterious variants in 27 chicken populations based on SIFT prediction

Category	Term	Pathway ID	Count	P-value
GO term biological function	Microtubule-based movement	GO:0007018	6	0.004910801
	cytoskeleton-dependent intracellular transport	GO:0030705	3	0.026430039
	GTP biosynthetic process	GO:0006183	3	0.017017792
	UTP biosynthetic process	GO:0006228	3	0.017017792
	CTP biosynthetic process	GO:0006241	3	0.017017792
	Cilium morphogenesis	GO:0060271	4	0.035292561
	Homophilic cell adhesion via plasma membrane adhesion molecules	GO:0007156	7	0.020793417
Go term molecular function	ATPase activity	GO:0016887	9	9.95E-04
	Microtubule motor activity	GO:0003777	6	0.002723165
	Serine-type endopeptidase inhibitor activity	GO:0004867	5	0.02212991
	4 iron, 4 sulfur cluster binding	GO:0051539	4	0.02383427
INTERPRO	Toll/interleukin-1 receptor homology (TIR) domain	IPR000157	6	2.09E-04
	Interleukin-1 receptor family	IPR015621	3	0.012953862
	Immunoglobulin-like fold	IPR013783	22	0.001598181
	Immunoglobulin-like domain	IPR007110	17	0.002214937
	Immunoglobulin subtype	IPR003599	13	0.012352987
	Kinesin, motor region, conserved site	IPR019821	4	0.04201322
	Serpin domain (SERine Proteinase Inhibitors)	IPR023796	4	0.018536739
	Serpin family	IPR000215	4	0.020916244
	Nucleoside diphosphate kinase	IPR001564	3	0.016977895
	C-type lectin fold	IPR016187	7	0.006188548
	Sushi/SCR/CCP	IPR000436	4	0.045649524
SMART	TIR	SM00255	5	0.001657044
	IG	SM00409	13	0.020118713
	SERPIN	SM00093	4	0.022489789
	NDK	SM00562	3	0.019548392
	CCP	SM00032	4	0.054729557

Summary of Go functions, pathways and processes showing functions related to health involved in innate antibacterial and antifungal immunity response and biosynthetic activity. ID = identifier, GO = gene ontology; SMART = Simple Molecular Architecture Research tools.

Table 4. Go terms enriched for non-synonymous deleterious variants in 10 chicken populations based on SIFT prediction

Category	Term	Pathway ID	Count	PValue
INTERPRO	WD40-repeat-containing domain	IPR017986	9	0.016272
GOTERM molecular function	Telomeric DNA binding	GO:0042162	3	0.021679
INTERPRO	WD40 repeat	IPR001680	8	0.02589
GOTERM molecular function	oxidoreductase activity	GO:0016491	5	0.025893
GOTERM cellular component	Lysosome	GO:0005764	5	0.02709
INTERPRO	WD40/YVTN repeat-like-containing domain	IPR015943	9	0.028383
GOTERM Biological function	DNA recombination	GO:0006310	3	0.028499
GOTERM cellular component	lamellipodium	GO:0030027	5	0.030521
GOTERM_BP_DIRECT	peptidyl-serine phosphorylation	GO:0018105	5	0.031163
GOTERM cellular component	external side of plasma membrane	GO:0009897	5	0.034197
GOTERM cellular component	centriole	GO:0005814	4	0.035157
GOTERM cellular component	neuronal cell body	GO:0043025	5	0.03812
GOTERM Biological function	DNA repair	GO:0006281	5	0.038616
GOTERM Biological function	positive regulation of cholesterol homeostasis	GO:2000189	2	0.041447
GOTERM molecular function	alpha-1,3-mannosyltransferase activity	GO:0000033	2	0.042633
INTERPRO	Sialidases	IPR011040	2	0.044212
GOTERM molecular function	ATP binding	GO:0005524	21	0.045558

Table 5. **Go terms enriched for non-synonymous deleterious variants with allele frequency greater than 0.9 in 27 chicken populations based on SIFT prediction**

Category	Term	Pathway ID	Count	P-value
GOTERM Molecular Function	Methyltransferase activity	GO:0008168	3	0.008812026
GOTERM Biological Process	Protein Autophosphorylation	GO:0046777	4	0.018881257
GOTERM Cellular Component	PML body	GO:0016605	3	0.036055331
GOTERM Biological Process	Replicative senescence	GO:0090399	2	0.036791597
GOTERM Biological Process	Positive regulation of DNA damage response, signal transduction by p53 class mediator	GO:0043517	2	0.048757466

Summary of Go functions, pathways and processes showing functions related to health involved in innate antibacterial and antifungal immunity response and biosynthetic activity. ID = identifier, GO = gene ontology

Discussion

Genomic diversity of indigenous chicken populations

Single nucleotide polymorphisms (SNPs) and other mutations may disrupt the RNA structure by interfering with the molecular function and hence cause a phenotypic effect (Sabarinathan et al., 2013). In this study, we performed whole genome sequencing for SNPs and used the identified SNPs to characterize genetic diversity in indigenous chicken populations of Ethiopia. About 21 million (n = 20,867,451) high quality SNPs were discovered in 27 populations (n = 284 birds). The 21 million SNPs discovered in this study was higher than the number of SNPs discovered in a previous study by Gheyas (2015) who reported 15 Million functional SNPs. The number of SNPs detected from individual population ranges from 10 to 12 million is higher than what is reported by Lawal who reported 5.8 million to 6.7 million for domestic chicken including two Ethiopian populations. Wide spread variations have been noted in chicken SNP density across chromosomes due to the variation in the nature of each chromosomes and assembly. The mean SNP density reported in this study are 21 SNPS per kb (± 5) or 1 SNP per every 48 bases is higher than the figure reported by Almas et al (2015) who reported15 SNPs/kb. From the total SNPs, about 28.12 % (5,868,599) of the SNPs are already reported in dbSNP (build 147) which currently contains ~21 million SNPs (n = 21,303,759) for chicken; while the rest of the SNPs (n = 14,998,852) are novel. In terms of the mean density per Kb discovered and the novel SNPs, this study reported comparatively in concordance with the previous study by Gheya *et al*., (2015) who reported 16 $\pm$ 10.01 SNPs/Kb. In contrast to the findings of Gheyas et al. (2015), macro chromosomes where found to have higher number of SNP density despite high recombination rate and in turn high SNP polymorphism in micro chromosomes (Burt, 2005). Even though, the high gene-density of the smaller chromosomes would make them susceptible to hitchhiking effects that could erode genetic variation, this effects appear to be offset by the far higher recombination rate of the micro-chromosomes (Aslam et al., 2012).

Much lower SNP density is found in sex chromosomes than autosomes as the high number of repetitive sequences mainly in W chromosome, whereas, the highest SNP density was reported for Chromosome 26 followed by chromosome 6 in spite of their lowest chromosomes size unlike what is reported by Gheyas et al. (2015). High density of SNPs where obtained for chromosome Z in the current reference genome (11 SNPs/kb compared to an average of ~3 SNPs/kb reported by Kranis et al. (2013) and 1.32 to 2.85 per kb by Ngeno and Khobondo (2017) for Kenyan indigenous chicken sex chromosome genome. This specific finding is in line with the statement of Wong et al. (2004) who stated that SNP density is independent of chromosome size except for chromosome 16. The second lowest SNPs/Kb detected from Chr31 could be mainly because of the partial representation of this chromosome in the current reference genome (Kranis et al., 2013). Our result, also is not in line with the findings of previous studies which reported reduced genetic variations on ChrZ than on autosomes for a multitude of potential reasons like low male effective population size due to skewed reproductive success among males, selective sweep due to selection on sex linked characters combined with lower recombination rates (Sundstrom, 2004). Despite, lower chromosome length, chromosome 16 was also found to have higher number of SNPs (21$\pm$5) though this chromosome is an exception to the characteristic of high recombination polymorphism rates in micro-chromosomes. This may be due to the presence of highly variable Major Histocompatibility (MHC) genes In comparison to autosomes, sex chromosomes have extremely much lower number of SNPs because of low polymorphism rate in the Z chromosome possibly as a function of multiple factors including skewed reproductive success among males leading to male effective population size, selection on sex linked characters and lower recombination rates

compared to autosomes (Sundstrom, 2004). Whereas, the W chromosome is the sex-limiting chromosome in chicken and its reduced genetic variability is the result of selection and the complete lack of recombination outside the pseudo autosomal region (Berlin and Ellegren, 2004; Moghadam et al., 2012; Wright et al., 2016; Xu et al., 2018). The total numbers of SNPs that overlapped with CEs are 3,859,925 (18% of the 21M SNPs), of which 2,619,665 are reported in dbsnp, while the rest are novel.

The proportion of SNPS that were found heterozygous (55.21%) for the overall chicken population (n = 284) is higher than the average heterozygous SNPs recommended (50%). The mean genome nucleotide diversity (π) of the entire chicken population is 0.02 $\pm$ 0.001 reported in this study lies in the range reported by Lawal (2018). Most birds have a characteristic division in chromosome size, with 5 or 6 large chromosomes, around 5 intermediate size chromosomes, and 25 to 30 very small chromosome pairs. The lowest average heterozygous SNPs are reported for Hugub chicken populations (about 40%). The local habitat of this specific population is Hot to warm semi-arid lowlands. The lowest heterozygous SNPS could be attributed to the fact that as the area is extremely hot and low density of the chicken population (narrow breeding base). The average transition to tranversion ratio of detected SNPs is 2.35 (Aslam et al., 2012). In another recent study, the transition and transversion (TS/TV) ratio for SNPs initially detected in the whole-genome of commercial layers and broilers was 2.17, while the ratio in the filtered set was 2.31 (Boschiero et al., 2018).

The expected Ti/Tv ratio of true novel variants can vary across the genome attributed to variability in the CpG and GC content of the genome. For instance, in the case of exomes, an increased presence of methylated cytosine in CpG dinucleotides in exonic regions leads to an increased Ti/Tv ratio due to an easy deamination and transition of a methylated cytosine to a thymine. It is also observed that GC content is higher in birds and mammals than in invertebrates. Observed Ti/Tv ratio in this study is lower than the findings from Alsam et al. (2012) (2.45). This finding is in contrast to the fact that birds have higher TS/TV ratio for owning smaller genome size and a higher GC percentage in bird genomes.

Principal component and admixture analyses suggest the presence of four ancestral gene pools across the populations. Close proximity of majority of the populations often reflected their geographic proximity. The clustering of these populations follows the geographical pattern where they are sampled from.

Functional annotation of genes in indigenous chicken populations

The functional information of these variants can help in prediction of phenotypes or genetic merit with higher accuracy and selection of individuals can be done accordingly. Annotation of 21M SNPs against ENSEMBL gene annotation database shows that 46.36 % of SNPs are located within genes (intronic + exonic + UTRs + splicing) and the rest are available outside genes (intergenic and up/downstream), while, only 1.6 % (n = 331,968) of the SNPs are in protein-coding regions (i.e. exonic). However, the study by Wong et al. (2014) showed that only ~37 % of the variants fell within genes with only 1.2 % fell within the coding regions. Non-coding RNAs (ncRNAs) are an important class of genes, responsible for the regulation of many key cellular functions (Cao, 2014; Frías-Lasserre and Villagra, 2017; Gardner et al., 2015). The highest number and percentage of putatively functional SNPs that are present in high frequency and were detected from 27 populations shows that these genes are adaptive.

SNPs in a coding region can be synonymous (do not result in a change in amino acid; selectively neutral) and non-synonymous. The synonymous and non-synonymous (AA-altering) number of SNPs are 190,041(0.48 %) and 100293 (0.98%), respectively. Whereas, the other AA altering variant, number

of stop gain/loss accounts for about 0.36% (n = 1,209). Even though non-synonymous SNPs change amino acid sequence within a protein, the effects are not always harmful or radical on protein function. Using SIFT, 21.9 % of the non-synonymous variants (n = 44, 553) were predicted as 'intolerant' (INTOL) having radical effect, 64.94% (135,917) were predicted 'tolerant' (TOL), whereas the prediction for other variants had low confidence level. Much higher SNP observed in density in Chr16 (probably because it contains highly variable MHC regions) and also in smaller chromosomes (chr25-33). Smaller chromosome may be gene rich and hence may have greater SNP density. Apart from the amino-acid altering variants, other potentially functional categories are also reported in this study, such as splicing variants (0.006%); variants in 3′and 5′ UTRs with possible roles of regulating protein translation (0.82 %); those within 1 kb up- or downstream of transcription start or end sites (3.06 %) with possible roles on transcriptional regulation; and finally, the SNPs belonging to ncRNAs (2.61%).

Genomic regions conserved across distantly related species are assumed to be under purifying selection, and hence variants within these regions are likely to be harmful (Gheyas et al., 2015). Hence, SNPs overlapping evolutionary conserved elements were checked as these may have potentially functional effects. The 21 million SNPs were annotated against 1.1 million conserved elements (CEs) across 48 birds plus a lizard. These CEs covers about 2.1% of chicken genome (total length of CEs is 186,488,363 bases). The total numbers of SNPs that overlapped with CEs are 3,859,925 (18% of the 21M SNPs), of which 2,619,665 are reported in dbsnp, while the rest are novel.

The allele frequency distribution of different annotation categories showed that the largest proportion of variants fell within the AAF bin of ≤ 10%. However, the proportion was higher for potentially harmful variants like deleterious missense and stopgain/loss (> 60%) compared to neutral categories like intergenic, intronic, and synonymous (< 40%). This is expected as potentially detrimental SNPs are expected to be mostly low frequency. However, contrary to our expectation, we did not find any variation in the AAF pattern of SNPs within CE category with potentially neutral variants. SNPs that are potentially function or deleterious but are present in high frequency (e.g. AAF > 0.9) is expected to have greater impact and may be under selection. Table 4 shows the number and percentage of putatively functional SNPs that are present in high frequency and were detected from 27 populations.

Go term enrichment analysis shows significant ($P \leq 0.05$) GO terms related to innate antibacterial and antifungal immunity response (IPR000157; Toll/interleukin-1 receptor homology (TIR) domain, IPR007110); Immunoglobulin-like fold and energy biosynthetic ((GO: 0016887; ATPase) activity, (GO: 0006183; GTP biosynthetic process), (GO: 0006228; UTP biosynthetic process), GO: 0006241; CTP biosynthetic process)). Toll proteins or Toll-like receptors (TLRs) and the Interleukin-1 receptor (IL-1R) superfamily are both involved in innate antibacterial antifungal, anti Prozozuan and anti-viral immunity in chicken, insects and in mammals (Blasius and Beutler, 2010, 2010; Cohen, 2014; Liao et al., 2010; Liu and Zhao, 2007; Ma et al., 2007). Interleukin-1 receptor family participate in the regulation of immune responses, inflammatory reactions, and hematopoiesis (Armant, n.d.; Beutler, 2004; Mukherjee et al., 2016; Takeda and Akira, 2001; Vasselon, 2002). Protein protease inhibitors constitute a very important mechanism for regulating proteolytic activity. In a similar fashion 385 nonsynonymous deleterious genes (352 reported) detected in 10 populations where functionally annotated and Go term enrichment analysis gave genes responsible mainly for DNA repair and binding (Go:0042162, Go:0006281), ATP binding (GO:0005524) and WD40 repeat domains (IPR017986, IPR015943, IPR001680). Annotation of non-synonymous deleterious SNPs with AAF > 0.9 and their functional characterization has confirmed genes attributed to Methyltransferase, Protein auto-phosphorylation, a class of nuclear body called promyelocytic leukemia (PML) which react against

SP100 auto-antibodies during viral infections; and a cell aging process associated with the dismantling of a cell as a response to telomere shortening and/or cellular aging and genes that controls positive regulation of DNA damage response, signal transduction by p53 class mediator.

Conclusions

This study confirms the existence of significant genomic diversity in indigenous chicken populations of Ethiopia, with most of the variants previously undescribed in commercial breeds.

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Candidate Signatures of Positive Selection in Ethiopian Chicken

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Abstract

Selective breeding for genetic improvement is expected to leave distinctive selection signatures within genomes. The identification of selection signatures can help elucidate the mechanisms of selection and accelerate genetic improvement. Ethiopia has several chicken ecotypes, which have evolved in different ago-ecologies. Here, we assess the footprints of candidate signatures of positive selection from whole genome autosomal sequences comprising 14,857,039 SNPs genotyped in Improved Horro, Local Horro, Hugub, Arabo and Jarso chicken populations of Ethiopia. We identified selection signals in 20 kb windows, with sliding steps of 10 kb based on estimators of pooled heterozygosity (Hp) and F-statistics (Fst). Selective sweep analyses using Hp and Fst identified genomic regions associated with production and reproduction. A total of 595 candidate genes showed high evidence of positive selection in indigenous chicken populations, including genes that were related to traits such as growth and egg production. Gene ontology analysis displayed several biological processes and KEGG pathways involved in oestrogen biosynthesis, nervous system development processes, calcium signaling, and biosynthesis of unsaturated fatty acids. The regions identified in this study are expected to provide genomic landmarks to enhance the ongoing breed improvement operations in improved Horro and for the other four chicken populations.

Keywords: Indigenous, Chicken, Improved Horro, Signature of selection, SNP

Introduction

Genetic makeup of populations is the result of a long-term process of adaptation to specific environments, ecosystems and of artificial selection. Selective breeding for genetic improvement is expected to leave distinctive selection signatures within genomes. The identification of signature of selection can help to elucidate the mechanisms of selection and accelerate genetic improvement by well understanding molecular pathways underlying phenotypic traits and breeding goals (Elferink et al., 2012). Selection leads to specific changes in the patterns of variation among selected loci and in neutral loci linked to them (Guo et al., 2016). These genomic foot prints of selection are termed as signatures of selection and usually used as to identify loci that have been subjected to selection. Various statistical approaches either the allelic frequency spectrum or the properties of haplotype segregation in populations are being used for detecting selection signatures at a genome wide scale (Qanbari et al., 2015a). Among others, pooled heterozygosity (Hp) statistic is a variability indicator based on allele counts across sliding windows of adjacent loci. The other commonly used statistic is the fixation index (FST) which measures the genetic differentiation based on variations in allelic frequencies among

populations (Qanbari and Simianer, 2014). The loci in the tails of the empirical distribution of Fst be used as candidate targets of selection (Akey, 2002). The evolution of new functions and adaptation to new environments occurs by positive selection, whereby beneficial mutations increase in frequency and eventually become fixed in a population (Tang et al., 2007). Local environmental adaptation and artificial selection can change the allele frequencies of specific loci: leading to a higher level of population differentiation (Fst) (Yang et al., 2014a). Adaptation, or positive natural selection, leaves an imprint on the pattern of genetic variation found in a population near the site of selection (Xue et al., 2009).

Local breeds make up most of the world's poultry genetic diversity, and are still very important in developing countries where they represent up to 95 percent of the total poultry population. These local breeds, which are well-adapted to extensive husbandry systems and suitable for resource-poor poultry farmers endowed with very limited means, should be thoroughly studied as a basis for enhancing their use and conservation (Besbes et al., n.d.). To understand phenotypic variation in farm animals and in poultry in particular, it is essential to define all potential genomic variation within a genome (Schmid et al., 2015). Discovery of genes with large effects on economically important traits has for many years been of interest to breeders (Wolc et al., 2014). In this study we used these statistics to detect signature of selection in improved and other indigenous chicken populations.

Recent advances in sequencing technologies have helped in the detection of candidate genome regions playing crucial roles in the evolution of production and reproduction traits in chicken. In this regard, various genes responsible for growth and egg production has been found. This study aimed to elucidate the effect of on-station improvement on signature of positive selection signatures through identifying selected candidate regions.

Materials and Methods

Experimental population description and breeding scheme for Improved Horro breed

A breeding program has been established in 2008 to improve productivity of Horro chicken, an indigenous population in the western highlands of Ethiopia. The breed improvement was established with the aim of making Horro chickens more productive in terms of egg number and body weight through selective breeding and maintaining genetic diversity. Breeding objectives are growth rate and egg production with a target of gaining 1500 gm for the former and 200 eggs/ hen/year for the later. The population was established from 3000 eggs purchased from two village market sheds in Horro. The pedigree descended from 26 cocks and 260 hens and were hatched and raised at the poultry research farm of DZARC (Dana, 2011). The base population had a wide range of morphologic and genetic diversity.

After the base population for each generation, 50 males and 300 females were selected to produce the next generation representing selected proportions of approximately 10-20% in the males and 50-60% in the females. Collected eggs are artificially incubated. All hatched chicks are checked for deformity, vaccinated (against Marek's at the hatchery, Newcastle at Day 1 and 21, Gumboro at day 7, Fowl pox in week 10 and Fowl Typhoid in week 14) , wing tagged, weighed and randomly assigned into pens of concrete floor filled with bedding material. The chicks are provided ad libitum with a standard chick (0-8 weeks: 20% CP and 2950 Kcal/kg of ME), grower (8-20 weeks: 18% CP and 2750 Kcal/kg of ME) and layer (21-onwards: 16% CP and 2750 Kcal/kg of ME) diet formulated at the centre. Birds in all age classes are provided ad libitum access to feed in the form of starter, pullet and layer ration and water.

The chickens are reared in a single deep litter house until 18 weeks of age under a standard housing space, with natural lightning after 8 weeks of age. Body weight and cumulative egg production are recorded on weekly basis. Males selected on their body weight at 16 weeks (BW16), and females are selected on their BW16 and on their cumulative egg production in 24 weeks after start of lay (EN24). From week 18 onwards, the selected males and all females are transferred to the layer house and kept in floor pens with 1 cock and 10 hens per pen. Pens are fitted with trap nests to facilitate full pedigree recording. Eggs are collected from selected hens for 10-12 days and incubated in three hatches to produce the next generation. For all hen body weights at 12 and 16 weeks of age are analysed, whilst, the cumulative egg numbers at weeks 8, 16 and 24 after onset of laying (Woldegiorgiss, 2015).

Sampling strategy, DNA extraction and sequencing

Chicken sampling strategy and blood sampling strategy; DNA isolation; DNA quality checking and library preparation; library QC and sequencing; mapping and variant calling steps and population structure analysis procedures has been outlined in the genome diversity chapter (Chapter3). PCA and other statistical procedures have been included in the same chapter.

Selective sweep detection

Selection sweep detection was carried out using Hp, and Fst statistics using VCFtools version 0.1.13 in an overlapping bin size of 20 kb and step size of 10 kb. These statistics involves comparing the average number of nucleotide differences from pair wise DNA sequences and the number of segregating sites. Using the pool heterozygosity (Hp) method (Rubin et al., 2010), the level of heterozygosity, were measured in the genome at a window of 20 kb and 10 Kb step size from 1-28 and 30-33 chromosomes. Signatures of selection in sliding windows was searched by determining pooled heterozygosity values (Hp) relying on the following equation:

$$Hp = \frac{2\sum n_{MAJ}\sum n_{MIN}}{(\sum n_{MAJ} + \sum n_{MIN})} \quad Equ(1)$$

Where $\sum n_{MAJ}$ and $\sum n_{MIN}$) are the sums of major and minor allele frequencies respectively for all the SNPs within each the 20-kb window. At each detected SNP position, we counted the number of reads corresponding to the most and least frequently observed allele (nMAJ and nMIN, respectively) for each population. The values for the Hp calculated for each window size were then subsequently Z-transformed using the equation:

$$ZHp = \frac{Hp - \bar{x}(Hp)}{\sigma(Hp)} = Equ(2)$$

Where μ is the mean and σ is the standard deviation of the Hp. Windows with large number of heterozygote SNPs show values above zero, they may reflect balancing selection signature. Only windows with at least 20 SNPs were extracted and set for analysis. From these windows, a genome-wide threshold score of Z (Hp) ≤ - 4.0 was considered following the steps used by Rubin et al. (2010). Population differentiation method (Fst) which compares differences in allele frequencies between individuals around the selected regions, were calculated for each SNP as described in Akey et al. (2002). Fst was calculated from the allele frequencies (not the allele counts) using the standard equation according to the principles of population genetics.

$$Fst = P_{i\ total} - P_i\ within \quad Equ\ (4)$$

Where, P_i within $= \frac{P(i)\ population\ 1 + P(i)\ population\ 2}{2}$ and $P_i = 1 - fA2 - fT2 - fC2 - fG2$ with fN being the frequency of nucleotide N (A, T, C or G), $P_{i\ total}$ is the total P_i for which allele frequencies in both populations are averages. The Fst values were Z-transformed as follows:

$ZFst = \frac{Fst - \mu Fst}{\sigma Fst}$ Z (FST) where,

Putatively selected regions were located in fully overlapping windows with an extremely low Fst /extremely high ZFst values (top 1 % level).

Gene ontology and pathway analyses

To establish the biological significance of the genes found within each candidate selected region, the genes putatively under selection were submitted to DAVID Bioinformatics Resources 6.8 (https://david.ncifcrf.gov/) for enrichment analysis of the Gene Ontology (GO) and the Kyoto Encyclopaedia of Genes and Genomes (KEGG) pathways (KOBAS version 3.0, http://kobas.cbi.pku.edu.cn/). All chicken genes that are annotated in Ensemble were used as the background set. The two analyses restricted over-represented genes to the Fisher exact P value < 0.05 default threshold.

Results

A total of 14857039 no of recalibrated autosomal SNPS were generated from 70 chicken samples of 5 independently clustered populations were used for downstream analysis of signatures of selection.

Principal component analysis

The genetic structure of both populations was examined on the basis of all available SNPs using the principal component analysis (PCA) based on (Figure 1). Accordingly, the 5 populations have showed a clearly defined genetic structure. The first two components accounted for 19.27 % and 7.1 % of the variation respectively. The suitability map of indigenous chicken populations have been mapped based on physico-climatic variables to check if the independent gene pools based on PCA overlaps their geographical adaptation. The following variables were considered for suitability mapping: Min temperature coldest month, precipitation seasonality, precipitation wettest quarter, precipitation driest quarter, % of cultivated Land, % of grass/scrub/woodland of total grid, proportion of crop rain fed or irrigated and carbon content (g/Kg).

Consequences of Hp variants detected

The different variant consequences are reported in Table 1. From the Zhp variants (ZHp < -4), the highest Hp variant is reported for Improved Horro compared to the other indigenous chicken population of Ethiopia (Table 1). For instance, the number of novel variants ranges from 4.6 % (Jarso) to 7.4 % (Improved Horro). There is no difference in the mean Hp between Improved Horro (0.33±0.058), Local Horro (0.33±0.058), and Hugub (0.33±0.064) chicken populations. The lowest mean Hp is reported for Jarso (0.29 ± 0.062) chicken population. A difference was not noted in terms of missense variants between Improved and Local Horro chicken population using Hp method. The minimum missense

variant is reported for Jarso population. SIFT prediction of Hp variants also shows the tolerated and non-tolerated deleterious Hp variants in the candidates of selection signature regions (Table 3).

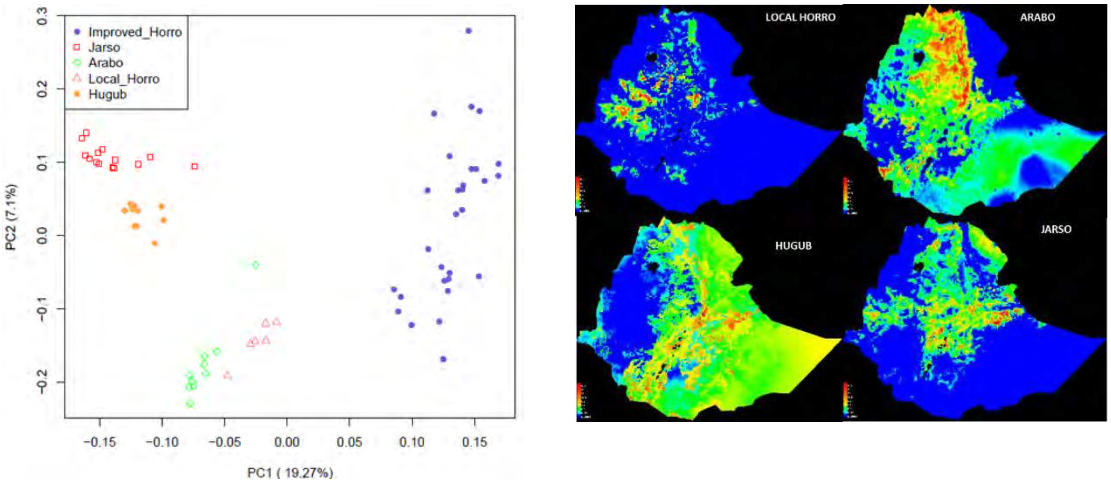

Figure 1. PCA plot of Improved Horro and other indigenous chicken populations and suitability map of other indigenous chicken populations (right) of Ethiopia.

Genome-wide selective sweep detection using Hp

Genome-wide SNPs restriction for Hp sweep detection was done as accuracy of sweep detection will depend on the number of SNPs in each window and considering the high polymorphisms identified within populations, windows with minimum of 20 SNPs were selected. Following this criterion, 1117, 1152, 1029, 1444 and 911 windows with SNPs < 20 were excluded from Improved Horro, Local Horro, Arabo, Hugub and Jarso populations respectively. Putatively selected genes were located by extracting windows that simultaneously presented extremely low ZHp (p < -04)) and extremely high ZFst (top 1 % level). These extremely low heterozygous regions were loaded to ensemble for further functional annotation. Significantly overrepresented GO terms and KEGG pathways (P < 0.05) among the candidate genes that are specific to each population was considered. The established selective sweeps around the TSHR gene in domestic chicken is identified in the five chicken populations and this was considered as a proof of principle demonstrated that the identification of selection signals using Hp methods is reliable. Myozenin 1(MYOZ1) gene is also found to be under the pressure of the ongoing selection program.

In Improved Horro chicken population, from the total number of 91996windows 90877 windows with 20 and above SNPs were analysed. The mean Hp value is reported as 0.33±0.058, while the minimum and maximum ZHp is -5.71 and 2.88, respectively. From the analysed windows, 417 windows passed the genome-wide threshold of ≤ -4 windows with ZHp. The proportion of windows with ZHp in Improved Horro (N=30) is 0.46% (417*100)/90878. 33,677 variants were processed using VEP and 2482 (7.4%)).These windows defined 417candidate sweep regions and 88 genes. Chromosomes 1 to15, Chromosome 17, Chromosome 20 and Chromosome 24 has depicted significant peaks ≤ -4). Across the genome, the strongest peak is located on chromosome 1and 12 (Chr1:189490000 to 189510000 bp; Chr12:190890000-190930000 bp regions) with a Z (Hp) score of -5.71variable genes including THSR gene (**Table 4**). Besides, the previously reported gene General Transcription Factor IIA Subunit 1 (GTF2A1) (Chromosome5: 40868271- 40894704 bp) is also under strong selection pressure in this population (Yuan et al., 2015a).

In Local Horro chicken population, from the total number of 91905windows 90753windows with 20 and above SNPs were analysed and of which, 311windows passed the genome-wide threshold of ≤ -4 windows with Zhp. The proportion of windows with ZHp is 0.34 % (311/90753*100). These defined 311 candidate sweep regions gave 68 genes. Chromosomes1 to 15, chromosome 17, chromosome 20 and chromosome 24 has depicted significant peaks ≤ -4).Across the genome, the strongest peak is located on chromosome 2, 3 and 5 (11 sweep regions) with a Z (Hp) score of -5.8. Similar to Improved Horro, but in a different region, the TSHR (Chromosome 5: 40858950- 40811286) and AGTR1 (Chromosome 5: 12430615 to 12398415) genes are available.

In Jarso chicken population, from the total number of 91, 966 windows 91055windows with 20 and above SNPs were analysed and of which, 31 windows passed the genome-wide threshold of < -4 windows with Zhp. Here, the proportion of windows with the threshold ZHp is 0.034 % (31/91055*100). Chromosomes 1 to 9 and chromosome 13 has depicted significant peaks ≤ -4). Across the Jarso genome, the strongest peak is located on chromosome 13 (520,000 to540000 bp; 530000-550000) with a Z (Hp) score of -4.68.

In Hugub chicken population, from the total number of 92006 windows 90560 windows with 20 and above SNPs were analysed and of which, 112 windows passed the genome-wide threshold of <-4 windows with Zhp i.e the proportion of windows with ZHp threshold is 0.12% (112/90560*100). The mean Hp value of the entire windows is 0.33±0.063. Whereas the minimum and maximum Z-transformed pooled heterozygosity value are -5.12 and 2.72, respectively. These regions yield 59 Ensemble genes (**Error! Reference source not found.**). Chromosomes 1 to 9 and chromosome 13 has depicted significant peaks ≤ -4). Across the genome, the strongest peak is located on chromosome 3 (50630000-50650000; 7020000-7040000; 7930000-7950000; 7940000-7960000; 18930000-18950000 bp) with a Z (Hp) score of -5.12. Another gene of biological interest on chromosome 3(26395277 to 26573746) from the Hugub chicken population Hp analysis is Protein Kinase C Epsilon (PRKCE).

In Arabo chicken population, from the total number of 92250 windows 91221 windows with 20 and above SNPs were analysed. The mean Hp value was (0.30± 0.061), while the z transformed minimum and maximum Hp value was -4.896 and 3.02, respectively. From the total windows analysed, 50 windows passed the genome-wide threshold of < -4 windows with ZHp. The proportion of windows with ZHp in Arabo is 0.055 % (50*100)/91221. These windows defined 56 windows with candidate sweep regions. Chromosomes 1 to 9 and Chromosome 13 has depicted significant peaks ≤ -4). Across the Arabo chicken genome, the strongest peak is located on chromosome 3 (82480000-82500000bp) with a Z (Hp) score of -4.896 and 14 other genes Tudor domain containing (TDRD), Diaphanous Related Formin 3 (DIAPH3), Ankyrin Repeat And KH Domain Containing 1 (ANKHD1), Steroid Receptor RNA Activator 1 (SRA1), phosphodiesterase 1C (PDE1C), Echinoderm microtubule associated protein like 4 (EML4), Regulating synaptic membrane exocytosis 1 (RIMS1), DLC1, Thyroid stimulating Receptor (TSHR) and Bone morphogenetic protein receptor type 2 (BMPR2) are under strong signature of selection. The TSHR (40811286- 40858950), a previously reported locus with known functions in metabolic regulation and reproduction process (Rubin et al., 2010) is reported, while, GTF2A1 known to be involved in the production of eggs in birds is not reported (Yuan et al., 2015 and Raman, 2018). GO enrichment analysis in Arabo chicken population indicates Caveola cellular component functions (Table 4).

Common selected Hp sweep regions across populations

The genome of the considered chicken populations were checked overlapping sweep regions. 655 regions were merged and checked for duplicate/overlapped regions. 145 duplicate values were obtained from the regions across populations. Finally, 64 regions were found overlapping regions between chicken populations. From these regions 31 genes were obtained for further functional annotation. Enriched functions for commonly selected regions include calcium signalling and other biological processes (Table 4).

Table 1. Summary of variant and Hp statistics in improved Horro and other Ethiopian indigenous chicken populations

Population	Improved Horro(n/%)	Local Horro	Arabo	Hugub	Jarso
Sample size	30	6	10	10	14
Single Nucleotide Variant	33677	26322	4358	20,281	2637
Novel variants	2482 (7.4)	1323 (5.0)	255 (5.9)	1202 (5.9)	121 (4.6)
Existing variants	31195 (92.6)	24999 (95.0)	4103 (94.1	19,079 (94.1)	2516 (95.4)
Overlapped genes	345	235	36	235	45
Overlapped transcripts	871	569	104	462	96
Windows Hp	91,994	91,905	92250	92004	91966
Windows greater than 20 SNPs	90,877	90,753	91221	90560	91055
µHp	0.33±0.058	0.33±0.058	0.30±0.067	0.33±0.064	0.29±0.062
minZHp	-5.71	-5.8	-4.9	-5.12	-4.68
Max ZHp	2.884263	2.880264	3.016422	2.724766	3.341756

N=sample size; SNV=Single Nucleotide variation; OG=Overlapped Genes; OT= Overlapped transcripts; WHp20>Windows greater than 20 SNPs; µHp=mean Hp;HpSD=standard deviation for pooled heterozygosity. Min ZHp=the minimum Z transformed pooled heterozygosity; Max ZHp=Maximum Zhp.

Table 2. Summary of all Hp consequences

Population	Improved Horro	Local Horro	Arabo	Hugub	Jarso
Missense	304	304	24	213	20
Splice region variant	328	244	9	95	35
Synonymous	1077	1000	64	513	108
5UTR	162	177	2	105	2
3UTR	1801	737	5	309	314
Non coding transcript exon	1034	556	33	299	109
Intron Variant	121102	77,505	21,622	40,194	8,386
Non coding transcript	10696	7,846	2,165	5,536	1,499
Upstream	8882	5,736	185	4,654	559
Downstream	11127	5,637	93	5,482	1,738
Intergenic	12671	11,567	2,105	9,788	1,169
Splice donor/acceptor	1(1)	0(3)			
Stop gain/lost	0(2)	4(1)		2(0)	
Micro RNA	1				

Upstream: a variant that is located in the 1-kb region upstream of the gene start site; stop gain: a non-synonymous (ns) SNP that leads to the creation of a stop codon at the variant site; stop loss: a non-synonymous SNP that leads to the elimination of a stop codon at the variant site; splicing: a variant within 2 bp of a splice junction; downstream: a variant that is located in the 1-kb region downstream of the gene end site; upstream/downstream: a variant that is located in the downstream and upstream regions of two genes.

Table 3. Sift prediction of Hp variants

Population	Deleterious low confidence	Deleterious	Tolerated low confidence	Tolerated
Improved Horro(N=30)	2	31	17	82
Local Horro(N=6)	1	23	6	176
Arabo(N=10)		7	2	14
Hugub(N=10)	4	14	19	97
Jarso(N=14)		12	6	

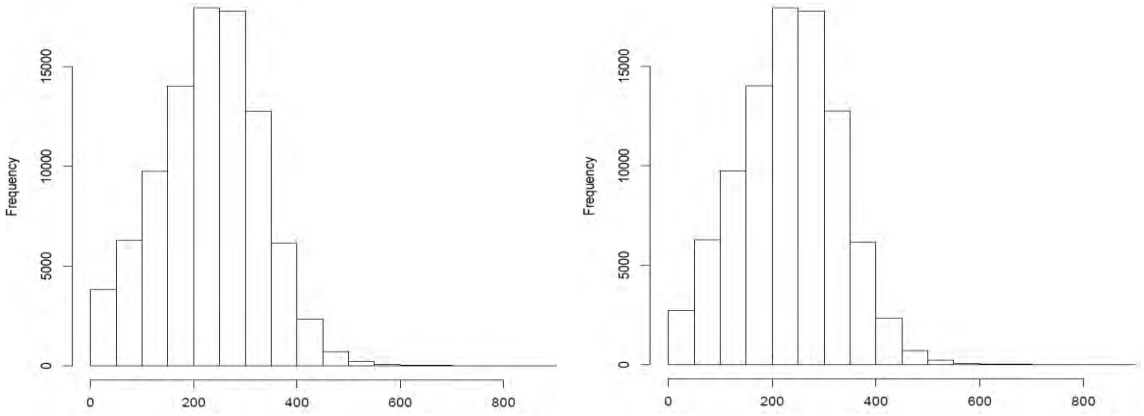

Figure 2. SNP count of Improved Horro (N=30) before and after filtering

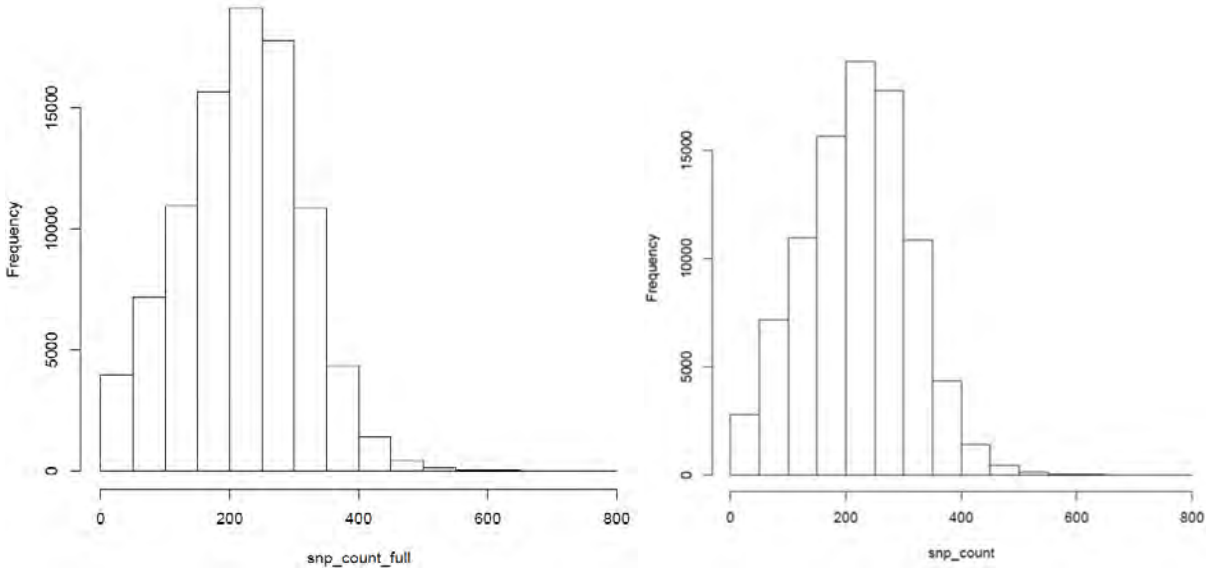

Figure 3. Frequency of ZHp values for and Manhattan plot in improved Horro (N=30)

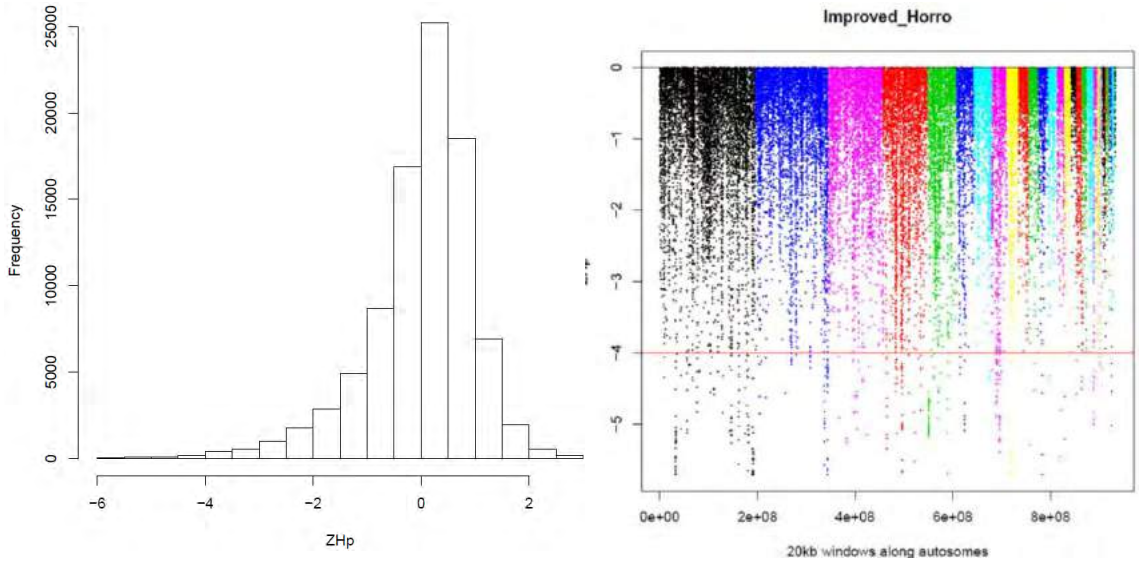

Figure 4. SNP count of Local **Horro (N=6) before and after filtering**

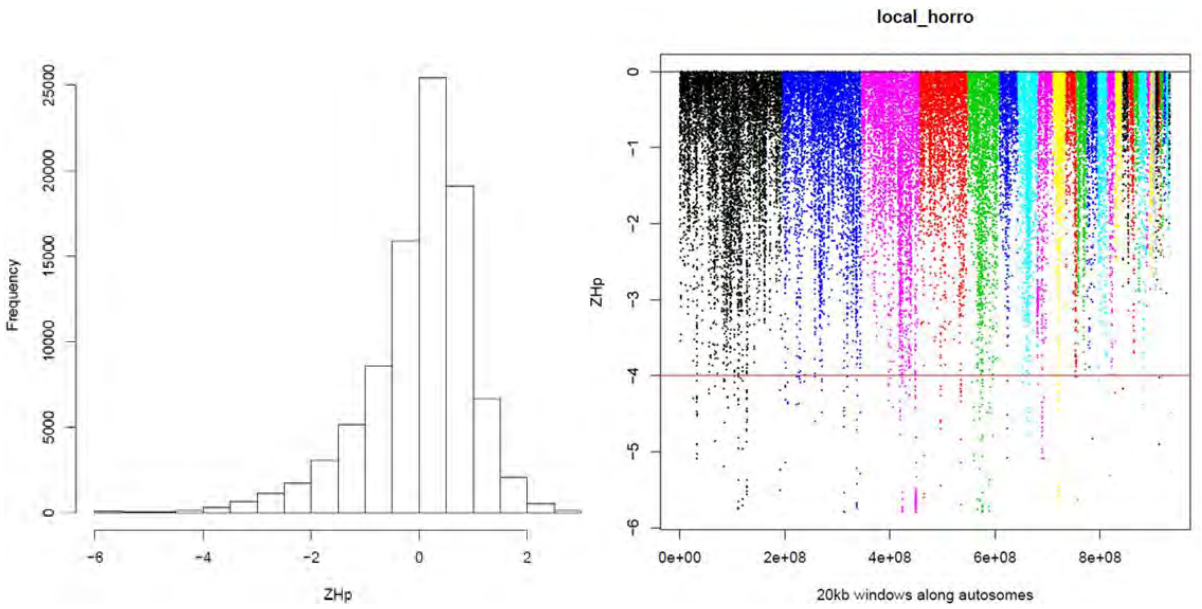

Figure 5. Frequency of ZHp values for and Manhattan plot in Local Horro (N=6)

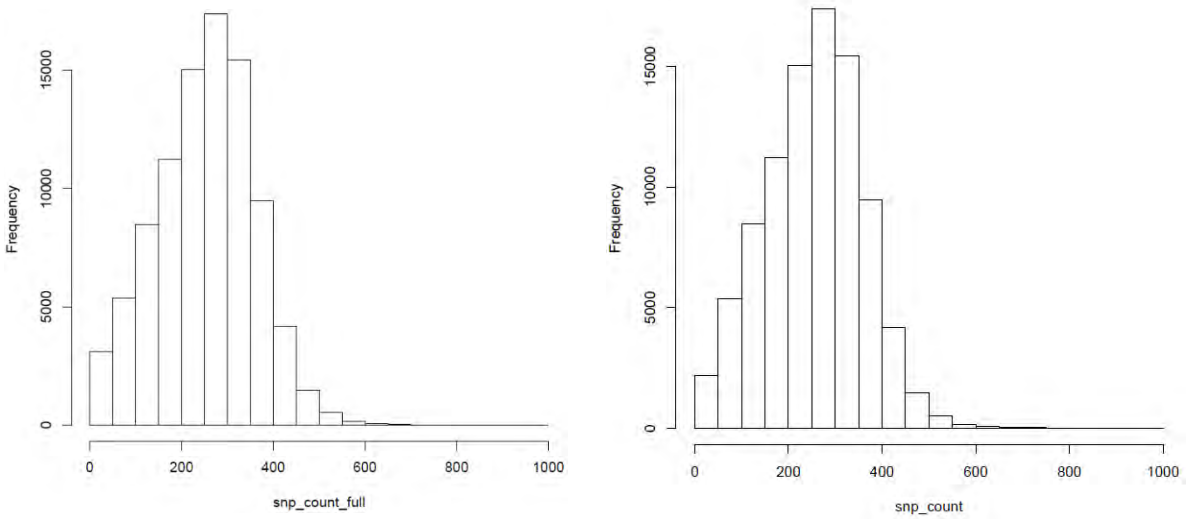

Figure 6. SNP count of Jarso (N=14) before and after filtering

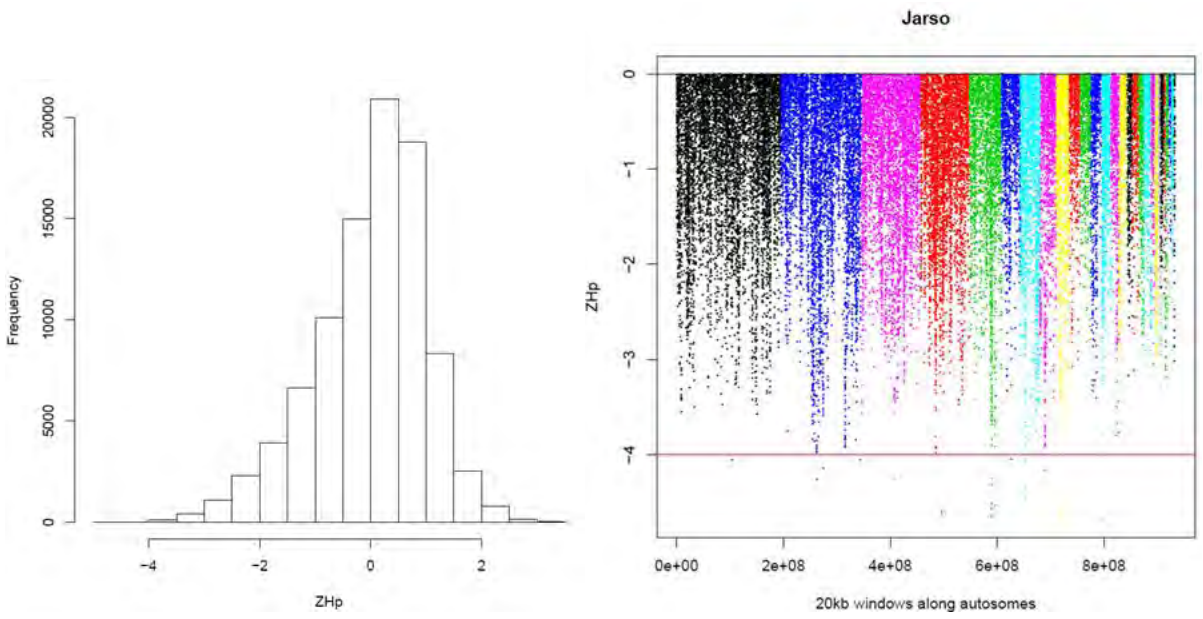

Figure 7. Frequency of ZHp values for and Manhattan plot in Jarso (N=14)

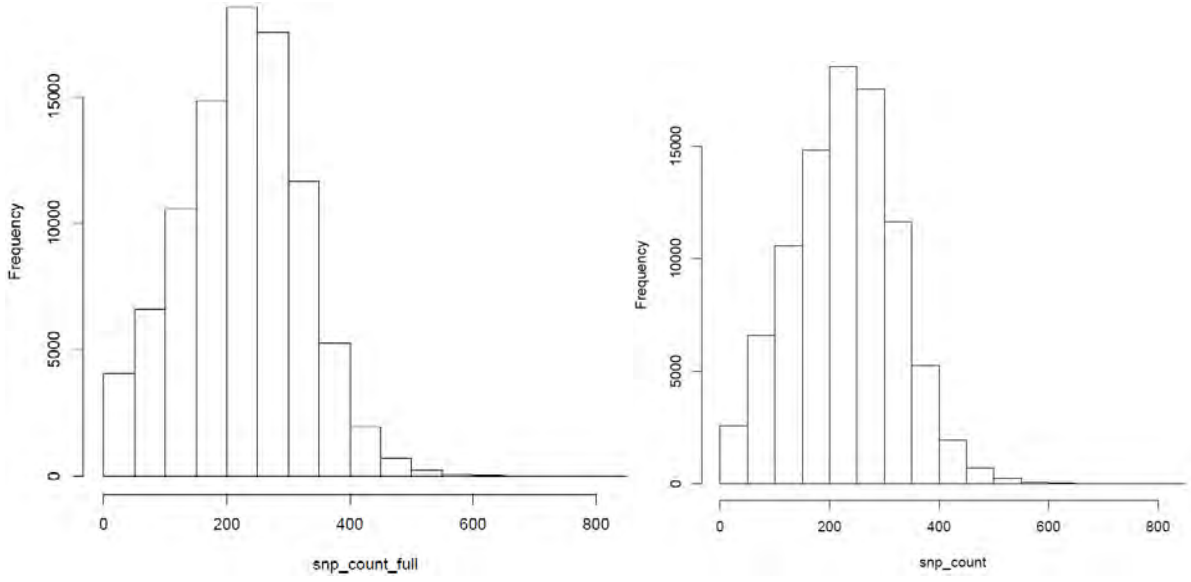

Figure 8. SNP count of Hugub (N=10) before and after filtering

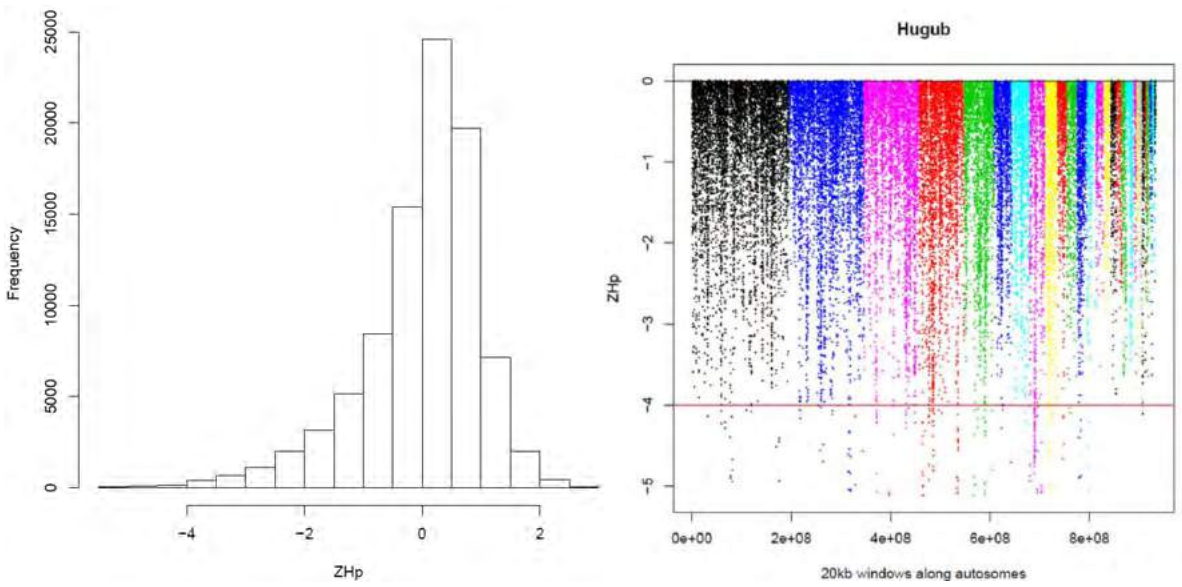

Figure 9. Frequency of ZHp values for and Manhattan plot in Hugub (N=10)

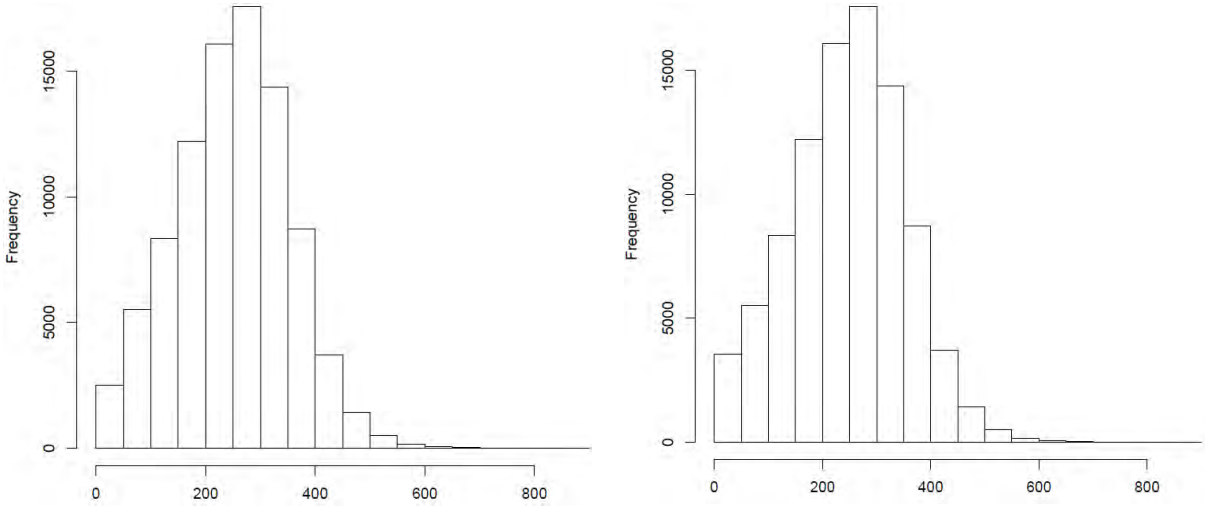

Figure 10. SNP count of Arabo (N=10) before and after filtering

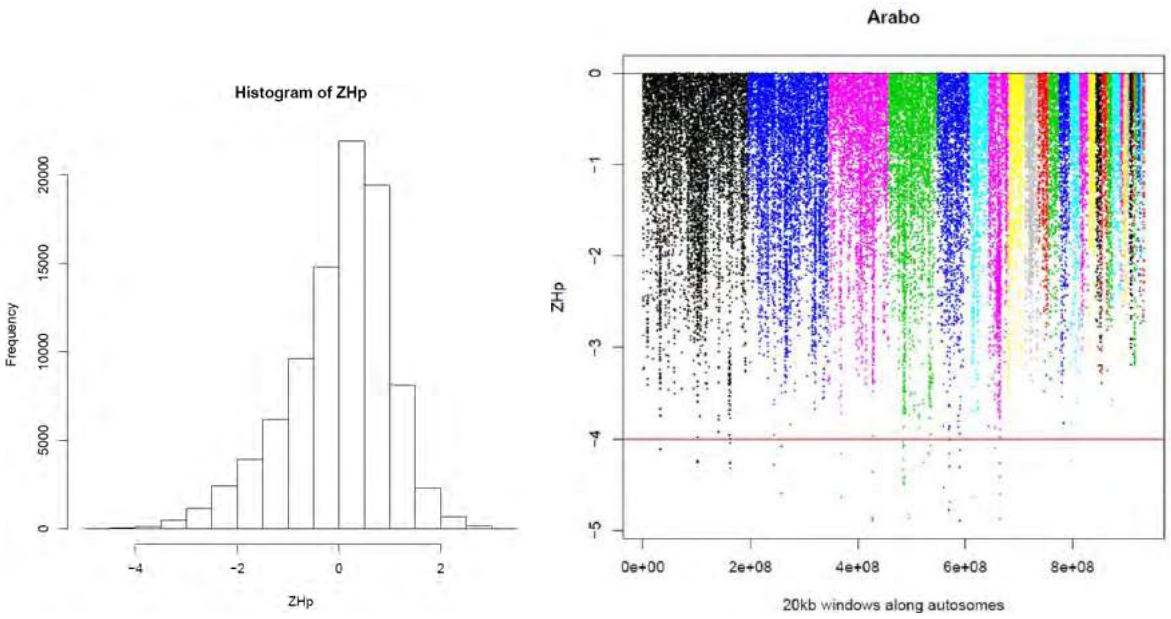

Figure 11. Frequency of ZHp values for and Manhattan plot in Arabo (N=10)

Consequences of Fst variants detected

The highest genetic differentiation is observed between the Improved Horro and Hugub chicken populations (0.55±0.06) and the lowest is between Improved Horro and Local Horro (0.08 ± 0.02) in the regions of the candidate of signature. The highest number of missense Fst variant is between Improved Horro and Local Horro implying that the ongoing selection is mainly targeting amino acid altering putatively functional variants involving non tolerable deleterious variants (Table 7).

Genome-wide selective sweep detection using Fst

Local environmental adaptation and artificial selection can change the allele frequencies of specific loci: leading to a higher level of population differentiation (Fst) (Yang et al., 2014b). Pair wise Fst results between Improved Horro and other indigenous chicken populations of Ethiopia namely Local Horro, Jarso, Hugub and Arabo chicken populations is given below.

From the total Fst windows (91,989) from Improved and local Horro 90,572 windows with greater than 20 SNPs the top 1% windows (906) were considered as significant windows for downstream analysis. The minimum and maximum Fst values were 0.04 and 0.15, respectively. The significant genetic differentiation based on Fst values was mainly concentrated in the majority of the chromosomes (Table 8). Detection of selection at the genome level using the Fst outlier method yield 311 candidate genes showing high evidence of positive selection from these significant regions. Between the highly differentiated regions of these populations an interesting gene called RALGAPA1 has been found. Signal transducer and activator of transcription 5b (STAT5b) gene is found candidate gene and it is associated with body weight and reproductive traits of Jinghai Yellow chicken (Zhou et al., 2005).

The number of windows in Improved Horro and Jarso population is 92123 windows. Windows with greater than 20 SNPs is 91395. The top 1 % significant windows where 914 windows and from these windows 191genes were extracted for further DAVID functional annotation.

The total number of Windows Fst for Improved Horro and Hugub populations is 92017. From this windows 91,167 number of windows have greater than 20 SNPs.These windows yield 207 Ensmeble genes. Syntaxin binding protein 6 (STXBP6) in chromosome 5, the gene responsible for bone allocation and fecundity trait is not found between Improved and Hugub chicken populations (Fu et al., 2016a).Chicken interleukin-21 (IL21) iscostimulatory for T cells and blocks maturation of dendritic cells. In mammals and chicken, interleukin-21 (IL-21) is an immunomodulatory cytokine with pleiotropic effects on the proliferation, differentiation and effector functions of T, B, NK and dendritic cells (Rothwell et al., 2012).

Table 4. Functional annotation of genes in Improved Horro and other indigenous chicken populations of Ethiopia

Improved Horro (N=30)				
Category	Term	ID	N	P<0.05)
GOTERM cellular component	Voltage-gated calcium channel complex	GO:0005891	3	0.004713343
KEGG_PATHWAY	MAPK signaling pathway	gga04010	6	0.005301658
KEGG_PATHWAY	Calcium signaling pathway	gga04020	5	0.010739355
GOTERM Molecular function	Low voltage-gated calcium channel activity	GO:0008332	2	0.017132492
INTERPRO	C2 calcium-dependent membrane targeting	IPR000008	4	0.02099244
INTERPRO	Laminin G domain	IPR001791	3	0.023680002
GOTERM cellular component	Cytoplasmic, membrane-bounded vesicle	GO:0016023	3	0.027615941
GOTERM Biological Process	Regulation of nucleic acid-templated transcription	GO:1903506	2	0.034513351
GOTERM Molecular function	Methylated histone binding	GO:0035064	2	0.045049799
GOTERM Biological Process	Calcium ion import	GO:0070509	2	0.048945698
Local Horro (N=6)				
GOTERM Biological Process	Nervous system development	GO0007399	3	0.028538
Hugub(N=10)				
Go term molecular function direct	Heparin binding	GO:0008201	3	0.040196
Jarso(N=14)				
GOTERM Biological Process	Adult locomotory behavior	GO:0008344	2	0.019311
GOTERM Molecular function	DNA binding	GO:0003677	3	0.032634
GOTERM Biological Process	Nervous system development	GO:0007399	2	0.038322
Arabo (N=10)				
Go term cellular component	Caveola	GO:0005901	2	0.017181377

The total number of windows for Improved Horro and Arabo population is 92118. 91300 windows have greater than 20 SNPs. From these windows a total of 913 regions have 1 % significant windows.179 genes were fetched from these regions. Insulin like growth factor 1 receptor (IGF1R). GRTP1- growth hormone regulated TBC protein 1(chicken). IGF2BP3 -insulin like growth factor 2mRNA binding protein 3(chicken). The protein encoded by the IGF2BP3 gene in human is primarily found in the nucleolus, where it can bind to the 5' UTR of the insulin-like growth factor II leader 3 mRNA and may repress translation of insulin-like growth factor II during late development. The encoded protein contains several KH domains, which are important in RNA binding and are known to be involved in RNA synthesis and metabolism. A pseudogene exists on chromosome 7, and there are putative pseudogenes on other chromosomes (https://pubchem.ncbi.nlm.nih.gov/target/gene/10643#section=Top). Integrin alpha-8/beta-1 (ITGA8) functions in the genesis of kidney and probably of other organs by regulating the recruitment of mesenchymal cells into epithelial structures (https://www.uniprot.org/uniprot/P26009).

Commonly selected Fst sweep regions across populations

The genome of the considered chicken populations were checked overlapping sweep regions.3644 regions were merged and checked for duplicate/overlapped regions.1652 duplicate values were obtained from the regions across populations. Finally, 667 regions were found overlapping. From these regions 190 genes were obtained for further functional annotation. It was evident that these selection signals mainly concentrated in macro and micro chromosome such as chromosomes 1 to 10.

Table 5. Fst variant Statistics

Population	IH VS LH	IH VS AR	IH VS JAR	IH VS HU
SNV	157,323	124,268	135913	108,541
Novel variants	19,232 (12.2)	16,450 (13.2)	19,855 (14.6)	13,850 (12.8)
Exiting variants	138,091 (87.8)	107,818 (86.8)	116,058 (85.4)	94,691 (87.2)
Overlapped genes	907	716	777	582
Overlapped transcripts	1,956	1,482	1,746	1,406
Fst windows >20 SNPs	90572	91300	91395	91167
Significant windows(P<0.01)	906	913	914	912
Min Fst	0.063628	0.404889	0.462838	0.475739
Mean Fst	0.085193	0.481478	0.534713	0.554283
SD	0.019528	0.079865	0.063902	0.06369
Max	0.184367	0.796025	0.760696	0.816078

IH=Improved Horro; AR= Arabo; JAR=Jarso; HU=Hugub

Upstream: a variant that is located in the 1-kb region upstream of the gene start site; stop gain: a non-synonymous (ns) SNP that leads to the creation of a stop codon at the variant site; stop loss: a non-synonymous SNP that leads to the elimination of a stop codon at the variant site; splicing: a variant within 2 bp of a splice junction; downstream: a variant that is located in the 1-kb region downstream of the gene end site; upstream/downstream: a variant that is located in the downstream and upstream regions of two genes.

Table 6. Fst all consequences

Population	IH Vs LH	IH Vs AR	IH Vs JAR	IH Vs HUG
Missense	3,185	1,473	1,797	2,375
Splice region	1,592	881	776	640
Synonymous	7,115	4,454	4,281	3,429
5UTR	1,478	1,719	1,788	666
3UTR	8,859	6,999	8,084	4,638
Noncoding Transcript Exon	4,802	4,617	5,652	4,135
Intron	43,352	41,695	50,499	36,617
Non-coding transcript	59,335	38,960	46,593	56,160
Upstream gene	66,220	44,935	44,749	29,015
Downstream gene	59,689	42,489	45,497	27,076
Intergenic	43,352	41,695	50,499	36,617
Splice donor/acceptor	10(9)	21(4)	20(19)	10(5)
Stop gain/lost	10(2)	5(4)	12(7)	5(1)
Micro RNA		3	1	1
Start lost / regained	5(4)			4

IH=Improved Horro; LH=local Horro; JR=Jarso; Hu=Hugub; 5UTR=5 prime untranslated region; 3UTR=3 prime untranslated region

Table 7. Number of F_{ST} variants based on SIFT prediction

Population	Deleterious low confidence	Deleterious	Tolerated low confidence	Tolerated
Improved Horro VS Local Horro	33	180	82	646
Improved Horro VS Arabo	23	124	43	390
Improved Horro VS Arabo	27	171	70	486
Improved Horro VS Jarso	29	127	89	532
Improved Horro VS Hugub	13	85	36	354

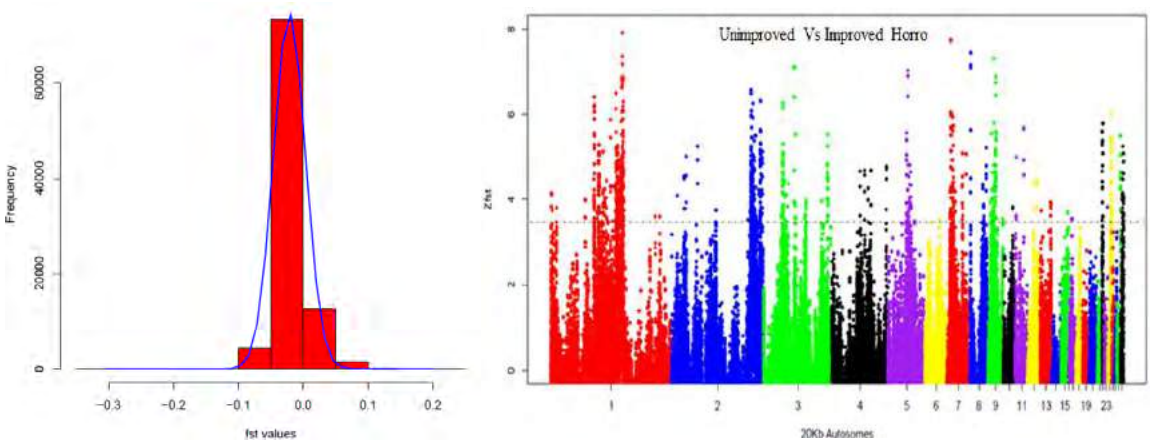

Figure 12. Histogram and Manhattan plot showing the distribution of Fst values for Improved Horro Vs Local Horro chicken populations

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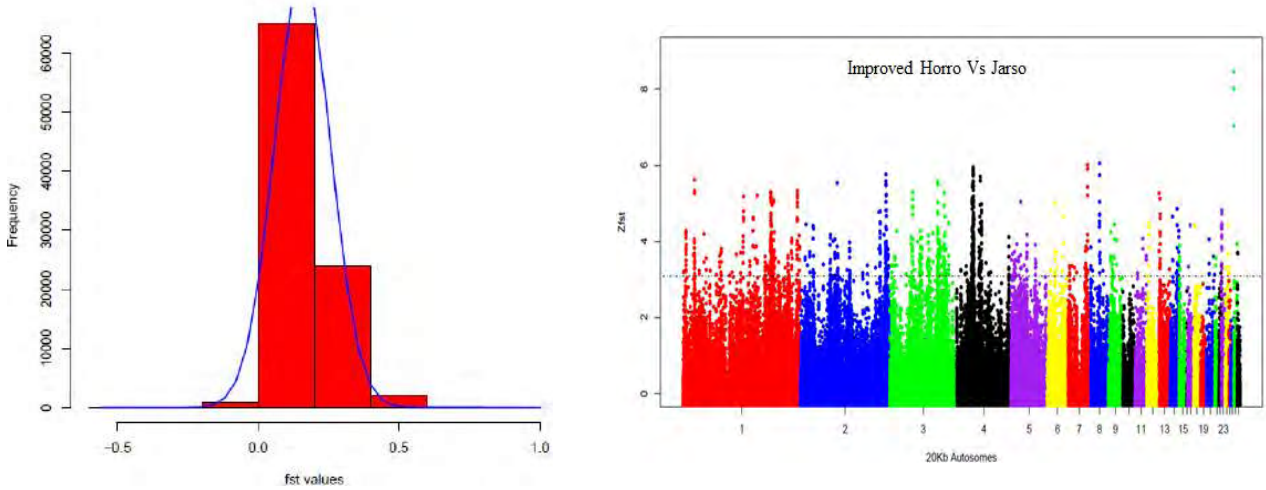

Figure 13. Histogram and Manhattan plot showing the distribution of Fst values for Improved Horro Vs Jarso chicken populations.

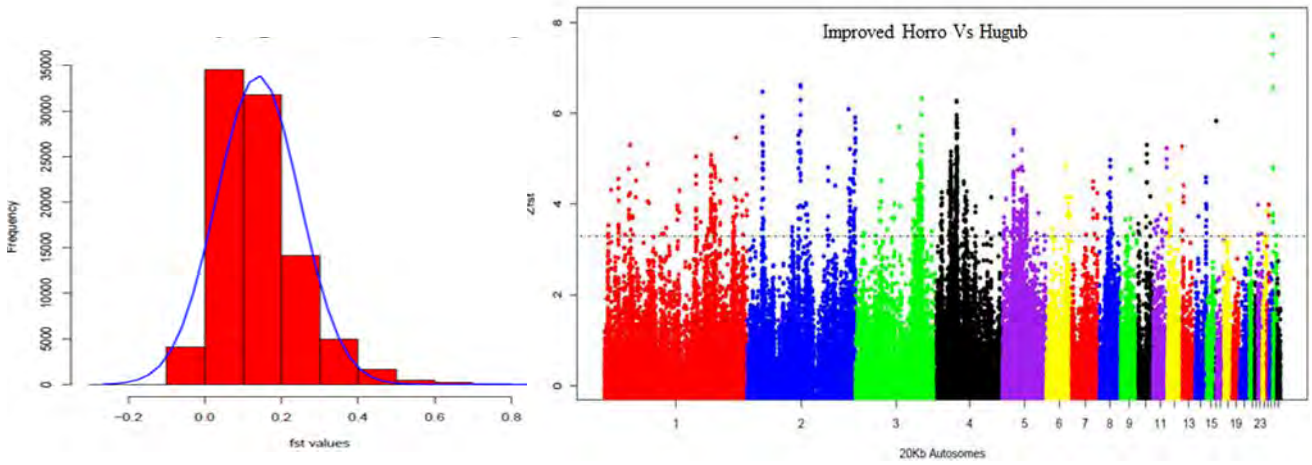

Figure 14. Histogram and Manhattan plot showing the distribution of Fst values for Improved Horro Vs Hugub chicken populations.

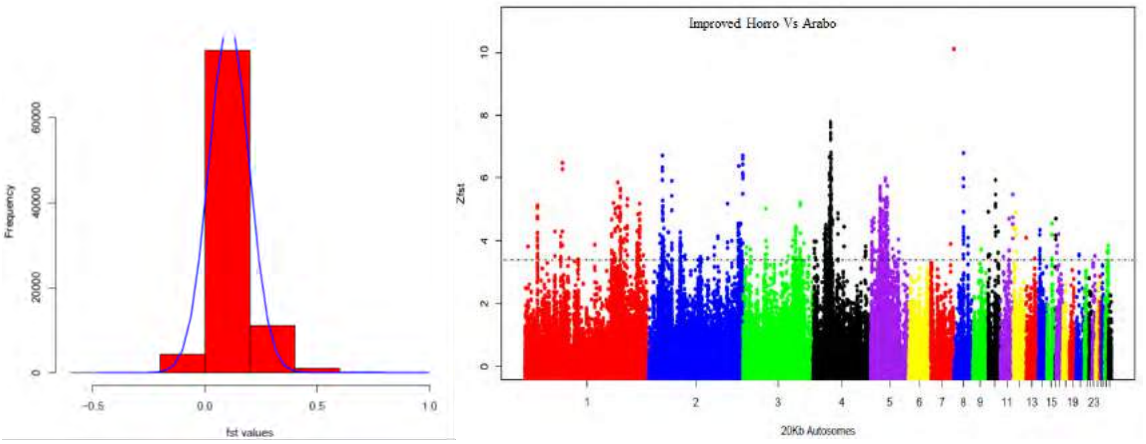

Figure 15. Manhattan plot for Ethiopian Improved Horro and Arabo chicken showing the pairwise comparison of selection pressure. Peaks on chromosome were analysed for selection pressure and were shown to be the two chromosomes on which the populations were the most diverged.

Table 8. Functional annotation of genes in Improved versus other indigenous chicken populations

Category	Term	ID	N	P<0.05
Improved versus Local Horro				
GOTERM Biological Process	Late endosome to vacuole transport	GO:0045324	2	0.023434
INTERPRO	Ankyrin repeat-containing domain	IPR020683	6	0.025137
GOTERM Biological process	Cell-cell adhesion	GO:0098609	3	0.04263
GOTERM Cellular component	postsynaptic membrane	GO:0045211	4	0.04394
GOTERM Biological process	Cellular response to fatty acid	GO:0071398	2	0.046323
GOTERM Biological Process	Positive regulation of CREB transcription factor activity	GO:0032793	2	0.046323
GOTERM Biological process	Protein transport	GO:0015031	4	0.047637
Improved Horro versus Jarso				
GOTERM Cellular component	Cell cortex	GO:0005938	4	0.016022
GOTERM Cellular component	Cleavage furrow	GO:0032154	3	0.016867
GOTERM Cellular component	Ruffle membrane	GO:0032587	3	0.036196
INTERPRO	Haemoglobin, beta	IPR002337	2	0.037098
INTERPRO	Ion transport domain	IPR005821	4	0.038218
Improved Horro versus Jarso … continue				
GOTERM Molecular function	GTPase activator activity	GO:0005096	5	0.040684

Category	Term	ID	N	P<0.05
INTERPRO	Potentiating neddylation domain	IPR005176	2	0.046158
INTERPRO	Defective-in-cullin neddylation protein	IPR014764	2	0.046158
INTERPRO	Cullin, conserved site	IPR016157	2	0.046158
GOTERM molecular function	Protein kinase activity	GO:0004672	4	0.046783
GOTERM molecular function	Ubiquitin-like protein binding	GO:0032182	2	0.04715
Improved Horro Versus Hugub				
GOTERM Biological process	hematopoietic progenitor cell differentiation	GO:0002244	4	0.004749
INTERPRO	Cadherin-like	IPR015919	4	0.016567
GOTERM cellular component	ciliary rootlet	GO:0035253	2	0.032998
GOTERM Biological process	Homophilic cell adhesion via plasma membrane adhesion molecules	GO:0007156	4	0.036298
GOTERM Biological process	Apoptotic process	GO:0006915	4	0.040854
GOTERM cellular component	Extracellular space	GO:0005615	10	0.055477
KEGG_PATHWAY	Gap junction	gga04540	3	0.079866
INTERPRO	Pleckstrin homology-like domain	IPR011993	6	0.084988
GOTERM cellular component	Interstitial matrix	GO:0005614	2	0.0958
Improved Horro and Arabo				
INTERPRO	Armadillo-type fold	IPR016024	8	0.017504
PIR_SUPERFAMILY	fatty acid desaturase/sphingolipid desaturase	PIRSF015921	2	0.019163
KEGG_PATHWAY	Biosynthesis of unsaturated fatty acids	gga01040	3	0.020228
INTERPRO	Zinc finger, FYVE/PHD-type	IPR011011	5	0.020317
INTERPRO	Fatty acid/sphingolipid desaturase	IPR012171	2	0.02156
INTERPRO	FAM122	IPR026716	2	0.02156
INTERPRO	Tetraspanin, EC2 domain	IPR008952	3	0.023362
GOTERM_BP_DIRECT	Estrogen biosynthetic process	GO:0006703	2	0.031684
GOTERM_BP_DIRECT	Positive regulation of axon regeneration	GO:0048680	2	0.042023
INTERPRO	Diaphanous autoregulatory	IPR014767	2	0.04266
INTERPRO	Zinc finger, N-recognin	IPR003126	2	0.04266
SMART	ZnF_UBR1	SM00396	2	0.04589

Table 9. Functional annotation of commonly selected genes based on Fst

Category	Term	ID	N	P<0.01
INTERPRO	Fatty acid/sphingolipid desaturase	IPR012171	2	0.014103
KEGG_PATHWAY	Fatty acid metabolism	gga01212	3	0.031962
INTERPRO	Cullin protein, neddylation domain, conserved site	IPR019559	2	0.041723
GOTERM Biological process	Positive regulation of G1/S transition of mitotic cell cycle	GO:1900087	2	0.045811
INTERPRO	Cullin homology	IPR016158	2	0.048508
INTERPRO	Fatty acid desaturase, type 1	IPR005804	2	0.048508

Discussion

Candidate of signals of selection using Hp method

Selective sweep can have a dramatic impact on the level of population subdivision, particularly when the sweep has not yet spread to all populations within a species (Nielsen et al., 2011).The size of a selective sweep may depend on factors such as the local recombination rate, whether the selected variant ever reached complete fixation, the number of generations it took before fixation and any population admixture at a time point after the sweep initially occurred (Rubin et al., 2010). Across the Improved Horro genome, the strongest peak is located on chromosome 1 and 12 (189490000 to 189510000 bp) with a Z Hp score of -5.71 including Thyroid Hormone Stimulating Receptor (THSR) and other variable genes (**Table 4**). The TSHR gene (Chromosome 5: 40811286-40858950 bp)which is a previously reported locus with a pivotal role in metabolic regulation and reproduction process (Rubin et al., 2010), is reported in Improved Horro chicken population. It is regarded as one of the most striking selective sweeps found in all domestic chicken. Besides, the previously reported gene (Chromosome 5: 40868271- 40894704 bp) known to be involved in the production of eggs in birds is also under strong selection pressure in this population (Lawal et al., 2018; Yuan et al., 2015b).

Among other numerous genes in this study, the previously reported ovostatin (OVST) gene which is associated with the formation of eggshells by regulating eggshell matrix protein secretion is also under strong selection (Cordeiro and Hincke, 2016). Angiotensin II Type 1 Receptor (AGTR1) (Chromosome 9: 12398415- 12430615 bp) gene is the other gene available in the selective sweeps of this specific population. This gene is also reported heavily involved in Ascites in commercial broilers (Krishnamoorthy et al., 2014). Ascites refers to abnormal accumulation fluid in the abdominal (peritoneal) cavity and it is a disease of modern days in the poultry industry (Qanbari et al., 2015b; Wideman et al., 2013). In humans, AGTR1 (Chromosome 9: 12398415- 12430615 bp) is a strong candidate for the pulmonary arterial hypertension (Burks, 2011; Chung et al., 2014; Crossley and Altimiras, 2012). The Immunoglobin Superfamily Member 21 (IGSF21) (Chromosome 21: 23092-65640 bp) which promotes differentiation of inhibitory synapses via binding to neurexin2α is also under selection pressure (Tanabe et al, 2017). Coordinated development of excitatory and inhibitory synapses is essential for higher brain function, and impairment in this development is associated with neuropsychiatric disorders. The most selected chromosome in Improved Horro is chromosome

1(**Error! Reference source not found.**; **Error! Reference source not found.**). Genes of interest that contain statistically significant includes the DnaJ heat shock protein family (Hsp40) member C12 (DNAJC12) (Chromosome 6: 6666081- 6675111 bp) in Improved Horro chicken population. The DNAJC12 gene plays a pivotal role in negative regulation of neuron apoptotic process (Fleming et al., 2017). Functional annotation of genes showed calcium signaling pathways and functions related to histone methylation (**Table 4**). Another interesting phenomenon is the presence of Myoz1 gene under the candidate signatures of selection which plays a crucial role in signal transduction and muscle fiber type differentiation. The Myoz1 gene is a potential candidate for affecting carcase and meat quality traits in animals (Luo et al., 2018).

Together with TSHR, GTF2A1, AGTR1 and many other genes, the BCDO2 (Chromosome24: 6130965 -6110301) gene is the only gene uniquely available in the candidate signature of selection in Local Horro. This gene is known to express in the skin where it encodes an enzyme that cleaves colourful carotenoids into colourless apocarotenoids, and polymorphisms in the BCDO2 gene have well-known effects on skin pigmentation in birds (Eriksson et al., 2008).To this end, it looks that the ongoing Improved Horro selection and improvement operation is working against this specific gene as it is not found in the selection signature regions of Improved Horro.

Unlike other populations Jarso chicken populations have fewer number of variants under strong selection of signature. In addition to the common genes TSHR and GTF2A1 many other genes are under strong selection signature. These include: amyloid beta precursor protein (APP), Ankyrin Repeat And KH Domain Containing 1 (ANKHD1), Glycerol Kinase 5 (GK5), Heat shock factor protein 2 (HSF2), Steroid Receptor RNA Activator 1 (SRA1), tachykinin receptor 3
(TACR3), Transcription factor Dp-2 (TFDP2), and T-SNARE Domain Containing 1 (TSNARE1) are also the genes under strong selection of signature in Jarso chicken populations.

Hugub chicken was also having the OVST gene together with TSHR and GTF2A1 genes. Protein kinase C epsilon (PRKCE) (Chromosome 3: 26395277-26573746) gene is also another gene of interest in Hugub chicken population. In humans, PRKCE is considered as a stress response gene involved in cardiac tissue (Fleming et al., 2017) however its impact is less defined in chicken. Considering the hot arid environment of the home habitat of the population the later function makes much more sense to justify the findings of this study. Enrichment of go term functions shows leucine rich regions involved in Heparin binding in Hugub populations.

Apart from, the TSHR (Chromosome 5: 40811286- 40858950 bp), and GTF2A1 (Chromosome 5: 40868271- 40894704) Arabo population also possess many other genes as a candidate signatures of selection. Ankyrin Repeat And KH Domain Containing 1 (ANKHD1, bone morphogenetic protein receptor type 2 (BMPR2), Diaphanous Related Formin 3 (DIAPH3), DLC1, echinoderm microtubule associated protein like 4 (EML4), Phosphodiesterase 1C
(PDE1C), regulating synaptic membrane exocytosis 1 (RIMS1), Steroid Receptor RNA Activator 1 (SRA1), and Tudor domain-containing protein 3 (TDRD3) are also the annotated genes under strong signature of selection in Arabo chicken populations. Functional annotation of signature of selection regions also show various functions like Caveola cellular component functions (Table 4). Caveolae are spherical invaginations of the plasma membrane and associated vesicles that are found at high surface densities in most cells, endothelia included. Caveolae are known to involve in many cellular functions such as endocytosis, signal transduction, mechano-transduction, potocytosis, and cholesterol trafficking.

Candidate of selection signals based on Fst method

Signal transducer and activator of transcription 5b (STAT5b), the gene responsible for bone allocation and fecundity trait is found between Improved and Local Horro (Fu et al., 2016b). Luo*et al*. (2018) states that STAT5b gene is associated with body weight and reproductive traits of Jinghai Yellow chicken. The only gene found in significant regions between these two populations is a gene called AGTR1 (Angiotensin II (Ang II)) which is an important regulator of cardiovascular function in adult vertebrates and have roles in thermoregulation (Crossley et al., 2010). This gene is known to heavily involve in Ascites in commercial broilers (Krishnamoorthy et al., 2014). The other gene is roundabout guidance receptor 2 (ROBO2) (96895939- 97053385 bp) gene which belongs to the immunoglobulin superfamily and plays functions associated in axon guidance and cell migration and are involved in SLIT/ROBO signalling (Wang et al., 2014). The ROBO2 gene, has a strong effect on the antibody response to the NDV in chickens (Luo et al., 2013).The RALGAPA1 (36275772-36390043 bp) gene which is known to play a pivotal role in reproductive traits and broodiness is also under strong selection in Improved Horro and Local Horro chicken populations (Shen et al., 2012).

A gene called unconventional myosin-VI; MYO6 (80736607-80807004 bp) which serve in intracellular movements are also found between the high confidence selection regions of Improved Horro and Jarso chicken populations. Myosin 6 is a reverse-direction motor protein that moves towards the minus-end of actin filaments. The gene of interest is ankyrin 2 (ANK2) (57097275-57432432 bp) which was reported by Fan *et al*. (2013) previously which Ankyrins play key roles in activities such as cell motility, activation, proliferation, contact and the maintenance of specialized membrane domains. Like the ROBO2 gene, the ROBO1 gene, is known to have a strong effect on the antibody response to the NDV in chicken (Luo et al., 2013). Another interesting gene in these populations is IL15 are T-cell growth factors potentially capable of enhancing cell-mediated immunity in vivo and plays a critical role in immune system function (Lillehoj et al., 2001). It is related cytokines that stimulate the activity and proliferation of T cells in mammals.

On top of syntaxin binding protein 6 (STXBP6), TBC1 Domain Family Member 30 (TBC1D30), TBC1, Domain Family Member 7 (TBC1D7) genes, candidate Fst signals between IH and Hugub population, also evidences the presence of stress related genes, Hypocretin (orexin) neuropeptide (HCRT) (Fleming et al., 2015). Among many other genes, Toll-like receptor 2 family member B (TLR2B) gene is under strong selection pressure in Improved Horro and Hugub chicken populations. Toll-like receptors (TLRs) are a group of highly conserved molecules that initiate innate immune responses to pathogens by recognizing structural motifs (Kannaki et al., 2010). In response to pathogen associated molecular patterns, TLRs induce the production of reactive oxygen and nitrogen intermediates, inflammatory cytokines and up regulate the expression of co-stimulatory molecules, subsequently initiating adaptive immunity (Ibid).

Among other genes, the insulin-like growth factor 1 receptor (IGF1R) and Insulin-like growth factor 2 mRNA-binding protein 3 (IGF2BP3) genes, which are necessary for formal growth (Rubin et al., 2010; Stainton et al., 2015; Yang et al., 2014a), is found between Improved Horro and Arabo chicken populations. Between these populations the myosin-binding protein C, cardiac-type (MYBPC3) and GATA binding protein 3 (GATA3) genes are under strong pressure of selection. MYBPC3 gene is known as an accessory protein of vertebrate striated muscle thick filaments that modulate cardiac muscle contraction (Carrier et al., 2015). Haplo-insufficiency for the transcription factor GATA3 leads to hearing loss in humans. It is expressed throughout the auditory sensory epithelium (SE) (Alvarado,

2009; Alvarado et al., 2009). Signal transducer and activator of transcription 5b (STAT5b) gene is found candidate gene and it is associated with body weight and reproductive traits of Jinghai Yellow chicken (Zhou et al., 2005). Integrin alpha-8/beta-1 (ITGA8) functions in the genesis of kidney and probably of other organs by regulating the recruitment of mesenchymal cells into epithelial structures. Candidate Fst signals between IH and Arabo population also evidences the presence of stress related genes, Hypocretin (orexin) neuropeptide (HCRT) (Fleming et al., 2017) in the candidate signature of selection region. The previously reported genes, TBC1 domain family member 7 (TBC1D7) and TBC1 domain family member 30 (TBC1D30) genes which are associated with hypothermia and stress are found in this population (Fleming et al., 2017).

Three functional genes (HPCAL4, TRITI and MYCL) which are reported in Horro and Jarso chicken populations by Lawal et al (2018) is not found in the candidate of selection region between these populations. The functional annotation analysis shows that the genes that displayed evidence of positive selection are mainly involved in the protein transport and other biological processes (Table 8).

Conclusions

Since most of the candidate genes identified in the present study are novel and have probably been under recent selection, they should be of great interest for future research. Neural crest Hypothesis Domestication (FGFR-I) and Gonadotrophin-releasing hormone I (GNRH-I) genes are not in the candidates of signature of selection in Improved Horro and other indigenous chicken populations of Ethiopia.

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Genetic Variation at LEI0258 Locus in Ethiopian Indigenous Village Chickens

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Abstract

Indigenous chickens are locally adapted to environmental challenges and provide subsistence to millions of farmers in Africa. However, their productivity remains low compared to exotic chicken strains. Efforts are being made to combine the local adaptation of indigenous chickens with productive traits of exotic chickens. Understanding the link between genetic diversity and environmental challenges is opening the door to marker-assisted breed improvement programs for sustainable chicken production at smallholder farmer level. Genetic variation at LEI0258 VNTR locus located within the MHC region has been linked to infectious diseases resistance/susceptibility in commercial breeds. Here, we report the diversity at LEI0258 in 236 chickens from 24 Ethiopian indigenous chickens populations from different agro-ecological zones. The number of alleles, allele frequency, and heterozygosity levels were used to measure ***genetic variation at LEI0258 locus in Ethiopian indigenous village chickens****. Twenty-nine LEI0258 alleles were observed using capillary electrophoresis, ranged from 185 to 569 bp with no significant difference in allele frequencies between populations (P < 0.01). Allele frequencies were in Hardy-Weinberg equilibrium in all populations except in Dara chicken. Excluding the tandemly repeated motif, we identified 412 monomorphic and 35 polymorphic sites. The numbers of point mutations and indels are 33 and 17, respectively. The number of R12 CTTTCCTTCTTT repeats ranged from 2 to 18, while R13 CTATGTCTTCTTT was found invariant in all populations. Sequence relationships revealed two distinct groups of alleles. The high diversity at LEI0258 in Ethiopian indigenous village chicken populations supports the importance of the MHC region in relation to the disease challenges faced by smallholder poultry production within and across Ethiopian agro-ecologies. We recommend that breed improvement programs ensure the maintenance of this diversity by selecting breeding stock as diverse as possible at the LEI0258 locus.*

Keywords: Chicken, Genetic Diversity, Ethiopia, LEI0258 VNTR, MHC

Introduction

High evolutionary pressures occurred in chicken during the course of domestication and subsequent natural and human selection (Downing *et al.*, 2009). Among others, infectious diseases exert strong selective pressures by affecting genes associated with innate and adaptive disease resistance and susceptibility. According to Salomonson *et al.* (2014) many genes involved in immunity are part of multigene families. In some families, each gene is conserved for a specific function dedicated to a particular outcome, in others allelic polymorphisms and copy number variations allow rapid evolution

in response to new environmental challenges, and there are also families comprising of both kinds of genes. The chicken Major Histocompatibility Complex (MHC) is one of these multigene families comprising loci encoding receptors which bind amino acid fragments from foreign pathogens on the surfaces of various immune and non-immune cells (Baelmans *et al.*, 2005; Chen *et al.*, 2012; Fulton *et al*., 2006; Fulton *et al.*, 2016; Jarosinski *et al*., 2010; Ncube *et al*., 2014; Nikbakht *et al*., 2013). MHC is a cluster of over 80 genes (92 kb) spanning chromosome 16 (Chazara *et al*., 2013, 2011; Nikbakht and Esmailnejad, 2015; Walker *et al*., 2011; Miller and Taylor, 2016) characterized by high polymorphism and a tight linkage into a single supergene complex , which are believed in association with disease resistance or susceptibility.

LEI0258 is a highly polymorphic variable number tandem repeat (VNTR) located near the BL/BF region of MHC-B locus on chromosome 16 (Kannak *et al*., 2017). This marker has been utilized as indicator of MHC diversity due to the low cost, easy access to detection technology and rapid results. The length variations of the LEI0258 are large and discrete, many alleles can be distinguished through agarose gel electrophoresis (Fulton, 2016). Although considered valuable, fewer limitations have been reported by different works like variation in instrumentation that can result in small size differences (1-6 bp). Similarly, different MHC -B serological defined and SNP defined haplotypes can have the same LEI0258 allele size (Fulton, 2006). Besides as it examines only one location of the MHC it fails to identify MHC recombinants. Another limitation of this VNTR marker was also best depicted in a study by Fulton et al. (2016), close examination of the SNP-defined haplotypes revealed distinct haplotypes that shared the same LEI0258 allele. However, currently typing the MHC based on the VNTR LEIo258 is the most used method to obtain genetic information on this region (Fulton et al., 2016). It has been reported to be associated with chicken performance and disease tolerance (Nikbakht and Esmailnejad, 2015), including allelic variation in antibody responses to vaccination against Newcastle Disease Virus (NDV) (Baelmans *et al*., 2005; Nikbakht *et al*., 2013), Marek's disease (Fulton *et al*., 2016; Wang *et al*., 2014), corona virus (Hateren *et al*., 2013) and coccidiosis, as well as its association with body weight, survival, embryonic mortality, fertilization rate, hatchability, egg production and resistance to worms (Owen *et al*., 2008). Given its high level of polymorphism and linkage disequilibrium with the MHC-B locus, LEI0258 genotypes have been suggested as an indicator of MHC-B haplotypes, and it have become an important genetic marker used in chicken breed improvement programs (Banat., 2013; Gao *et al*., 2015; Hoque *et al*., 2011; Weigend *et al*., 2001). This has been confirmed by Chazara (2013) who have ascertained that the LEI0258 marker genotypes an excellent predictor of the heterozygosity at the MHC locus.

LEI0258 is described as an atypical VNTR marker which is composed of 12 bp (CTTTCCTTCTTT) and 13 bp (CTATGTCTTCTTT) conserved repeat sequences which are flanked on both sides by indels and SNPs (Lima-Rosa *et al*., 2005; Fulton *et al*., 2006; Chazara *et al*., 2011). In an association study of MHC haplotypes with Marek's disease, Bumstead (1998) found that 96.5% of the birds with B-21 haplotype were resistant to viral infection while birds with B-19 haplotype suffered 100% incidence of mortality. Fulton *et al*. (2006) observed allele size diversity ranging from 182 bp to 552 bp. Lwelamira (2008) genotyped two chicken ecotypes from Tanzania using LEI0258 marker and identified 23 alleles. They further reported that allele of 206 bp had significant positive correlation ($P < 0.001$) with elevated antibody responses against NDV vaccine, whereas allele of 307 bp was positively correlated with body weight trait.

Indigenous chicken (IC) (*Gallus gallus domesticus*) are widely distributed in the diverse agro-ecological zones of Ethiopia. Accordingly, they represent ecotypes which may possessed unique combinations of

alleles in a set of given gene (Ngeno *et al*., 2015). Relatively, few works have been done so far on the genetic characterization of Ethiopian indigenous chicken. In particular, no study has attempted so far to characterise the immune system of Ethiopian chickens. We report here the characterization and diversity of the MHC-linked LEI0258 VNTR marker in 236 indigenous chickens from 24 distinct populations sampled across Ethiopia.

Materials and Methods

Whole blood sample collection

Blood samples were collected from 24 chicken populations in Ethiopia (Figure 1). Samples included 80 cocks and 156 hens. Except for the improved Horro, Gondar Zuria and Enderta populations, two villages per population were sampled (10 chickens from each village). One or two chickens were sampled per household. Improved Horro, the 8th generation breeding stock, was sampled at Debre Zeit Agricultural Research Centre (EIAR). Photographs and weight of each bird were taken. The average weight of sampled chicken was 1.26 Kg with age ranges of 5 to 36 months. Sampling included chickens from different agro-ecological zones with altitude ranges of 730-3500 meters (Table 1). From the wing vein of each chicken, 50 - 250 µl of whole blood were drawn with syringes using cryo-tubes filled with 1.5 ml absolute ethanol (100%) following the guidelines available at https://www.sheffield.ac.uk/nbaf-s/protocols_list.

Table 1. Information of sampling sites and number of samples per site.

No.	Sampling site	Sample size	Agro-ecology	Elevation (mabsl)
1	Adane	10	Tepid to cool moist mid highlands	2455
2	Alfa Midir	10	Cold to very cold moist sub afro-alpine to afro-alpine	3404
3	Amesha Shinkuri	10	Tepid to cool moist mid highlands	2464
4	Arabo	10	Tepid to cool moist mid highlands	1521
5	Ashuda	10	Hot to warm sub-moist lowlands	2028
6	Batambe	8	Tepid to cool moist mid highlands	2511
7	Bekele Girisa	10	Tepid to cool sub-humid mid highlands	1643
8	Dikuli	10	Hot to warm sub-moist lowlands	2093
9	Gafera	10	Tepid to cool moist mid highlands	2515
10	Gesses	10	Hot to warm sub-moist lowlands	1192
11	Gijet	10	Hot to warm sub moist lowlands	2050
12	Hadush Adi	10	Hot to warm sub moist lowlands	1494
13	Hugub	10	Hot to warm arid mid highlands	737
14	Kefis	10	Hot to warm moist lowlands	1061
15	Kido	10	Hot to warm sub-moist lowlands	1304
16	Kumato	10	Hot to warm sub humid lowlands	1728
17	Loya	10	Tepid to cool humid mid highlands	1896
18	Meseret	10	Hot to warm sub moist lowlands	2291
19	Metkilimat	10	Hot to warm sub moist lowlands	1751
20	Mihiquan	9	Tepid to cool sub-moist mid highlands	1316
21	Negasi Amba	10	Cold to very cold moist sub afro-alpine to afro-alpine	3060
22	Shubi Gemo	10	Tepid to cool sub-humid mid highlands	1555
23	Surta	9	Tepid to cool moist mid-highlands	2529
24	Tsion Teguaz	10	Hot to warm sub moist lowlands	1931

mabsl = meters above sea level

DNA isolation

Total DNA was extracted from chicken whole blood at the BecA-ILRI Hub, Nairobi, Kenya facility (http://hub.africabiosciences.org/) using the Qiagen DNeasy blood and tissue kit protocol (Lwelamira *et al*., 2008). To evaluate the DNA concentration the Thermo Scientific NanoDrop spectrophotometer 2000c was used. The integrity of DNA was confirmed by agarose gel electrophoresis whereby 20 ng/µl genomic DNA samples were loaded with 1 µl loading dye (6X) on 1% agarose gel containing 2.5 µl gel red at a voltage of 7/cm for 60 minutes, 3 µl of lambda DNA of size of 48,500 bp and at concentration of 20 ng/µl were used as size marker. The gel was then examined using UV light by the GelDoc-It^2 Imager to check the DNA quality and quantity. The DNA was normalized to 20 ng/µl using milliQ water for polymerase chain reaction (PCR) and genotyping.

MHC genotyping

PCR amplification

PCR amplification was carried out using the thermo-cycler PCR machine ABI PCR 9700 (Applied Biosystems). The primer sequences (GenBank accession number Z83781) for PCR amplification of LEI0258 were: forward 5'-CACGCAGCAGAACTTGGTAAGG -3' (length = 22 bp; GC content 47.6%; Tm = 71.5℃) and reverse-5'-AGCTGTGCTCAGTCCTCAGTGC-3' (length = 22 bp; GC content 46.2%; annealing temperature 69.9℃). The optimal PCR conditions were as described in (Gupta *et al*. (n.d).; Han *et al*. (2013); Izadi *et al*. (2011); Nikbakht *et al*. (2013) either in a total volume of 10 µl including 2 µl of template genomic DNA (40 ng), 5 µl of non-dyed *Taq* DNA Polymerase (1000 U) (Shangai, China), 0.3 µl of PET-labelled forward primer (3 µM), 0.3 µl of reverse primer (3 µM) and 2.4 µl of milliQ water or in a total volume of 50 µl including 3 µl of 20 ng template genomic DNA, 25 µl of Dyed Bioneer Master mix (2x), 3 µl of forward primer (3 µM), 3 µl of reverse primer (3 µM) and 16 µl of milliQ water. The PCR conditions were set with an initial denaturation at 94℃ for 3 minutes; 30 cycles of 94℃ for 45 seconds, annealing temperature of 63℃ for 1 minute and extension of 72℃ for 1 minute; final extension at 72℃ for 20 minutes and final hold at 15℃. Two microliters of PCR product were loaded on a 2% agarose gel containing 2.5 µl of gel red and separated by electrophoresis at a voltage of 7/cm for 60 minutes. A 1 Kb ladder DNA from Bioneer was used as a reference to the size of the amplicons (**Figure 1**). The gel was exposed to UV light using GelDoc-It^2 Imager, to reveal the amplified fragments and their sizes.

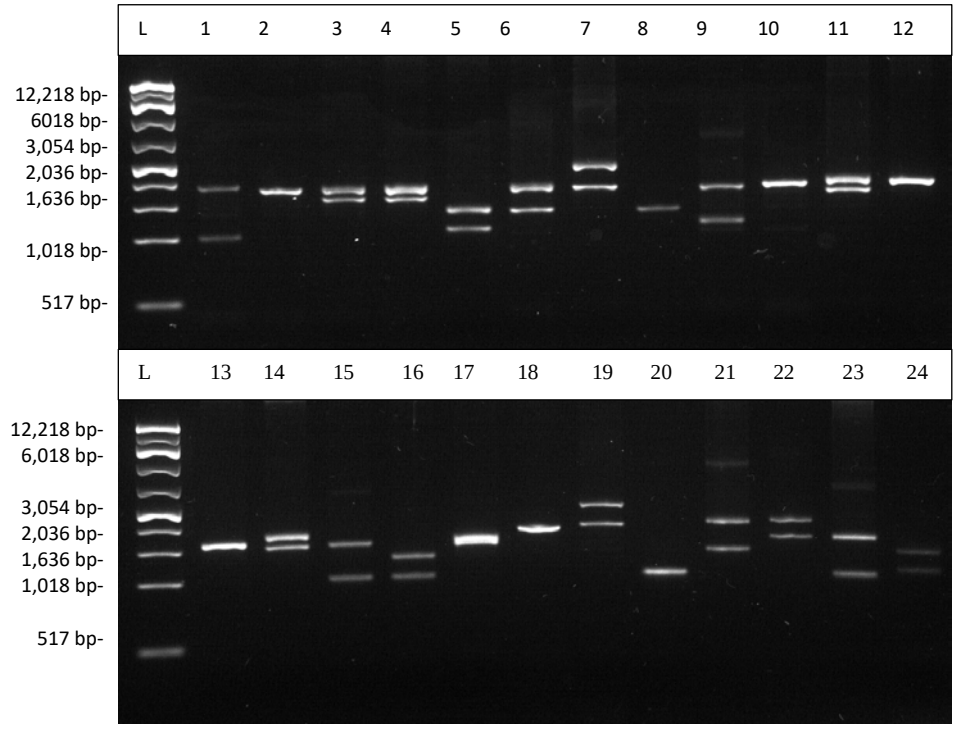

Figure 1. Image of alleles on 2% agarose gel run at 7/cm for 60 minutes in indigenous chicken populations of Ethiopia. 1 kb ladder DNA was used as a reference.

Capillary electrophoresis and sequencing

PCR product amplified using PET-labelled forward primer was added to a mixture of 12 µl GeneScan 500 LIZ® Size Standard and 1,000 µl of HIDI formamide, denatured at 95℃ for three minutes and separated by capillary electrophoresis using the ABI3730 DNA genetic analyzer (Applied Biosystems, Foster City, CA). The fragment (allele) sizes generated were scored with the GeneMapper Software Ver 4.1 (Applied Biosystems, Foster City, CA, USA) and exported to Microsoft excel for preparation of input files for statistical analyses. To confirm the polymorphisms from genotyping by capillary electrophoresis, PCR products of some homozygote and heterozygotes, were selected for the Sanger sequencing. The same primers used for genotyping were employed except that they were tailed with T7 (20 base pair) and SP6 (17 bp) primers for the forward and reverse primers, respectively. Homozygote DNA fragments were purified using GeneJet PCR purification Kit (Thermo Fisher Scientific; cat. No. K0701) while heterozygote DNA fragments were purified using the Qiagen Gel Extraction Kit and sent for sequencing to BIONEER sequencing platform in Korea. Alleles were sequenced on an ABI 3730XL DNA Analyzer using T-7 and SP6 primers. LEI0258 amplicons were sequenced in both directions as it provides confirmation of sequence and allow sequence information right up to the primer binding region.

Data analysis

LEI0258 locus diversity analysis. The genotypic data were subjected to various within and among populations genetic diversity analyses. These included: calculation of the total number of alleles, allelic frequency and their distribution among the entire populations, polymorphic information content (PIC) for each population, Shannon's Information Index using the GenAlEx software package version 6.5 (Peakall and Smouse, 2012). Observed heterozygosity (*Ho*), expected (*He*) heterozygosity was

estimated using the formula: $He = 1 - \sum (p^2_i + q^2_i)$ where p is the allelic frequency of the allele one at a given locus and q is the frequency of the alternate allele at the same locus (Ncumbe *et al*., 2014). The deviation of each population from Hardy-Weinberg Equilibrium (HWE) was also tested using GenAlEx software package. Number of homozygote and heterozygote genotypes were calculated using power marker analysis (Liu *et al*., 2002).

Sequence read data management and analysis: In addition, high quality sequence reads with base call accuracy higher than 95% were assembled and resolved for conflicts using Qiagen's CLC work bench version 7. The resulting consensus sequences of chicken populations were aligned using ClustalW program integrated in the MEGA (Molecular Evolutionary Genetics Analysis) software version 7 (Kumar *et al*., 2016). For this, a reference homologous sequences of LEI0258 marker (accession no. DQ239495.1) with SNP position was downloaded from the National Center for Biotechnology Information (NCBI).

Results

LEI0258 locus diversity based on sampling sites and MAEZs

We identified 29 LEI0258 alleles (100 genotypes; 64 heterozygotes and 36 homozygotes) in 24 populations. The effective number of alleles per population ranges from 4.0 (Hugub) to 11.76 (Arabo). Observed heterozygosity values ranges from 50% (Shubi Gemo) to 100% (Ashuda, Meseret, Gesses, Arabo and Hadush Adi). Size and frequency of the alleles are presented at **Table 3 and Table 4**. Private alleles are found in Ashuda, Meseret, Loya and Hadush Adu populations although at low frequencies (**Table 2**).

Across populations, the highest allele frequency and gene diversity are 16.31% and 91.9%, respectively, while they are 15.73% and 92.62%, respectively, across MAEZs. Two private alleles, of 315 bp and 385 bp are present in Surta populations while four private of 185 bp, 411 bp, 277 bp and 465 bp are found in Meseret, Ashuda, Loya and Hadush Adi populations 5%, 10%, 5% and 5%, respectively. The most frequent allele across populations is 315 bp (n = 77) followed by 197 bp (n = 57; Table 3). An average of 2.06 Shannon index is obtained across populations. The overall mean Ho among the entire population is 82.1%., with He ranging from 50% in Shubi Gemo to 100% in Arabo, Gesses and Hadush Adi populations.

Allele of 363 is present in all populations and in all MAEZs except in A1 and M1. The percentages of polymorphic loci are 91.38% and 92.16% across sampling sites and MAEZs. Another private allele of 185 bp is only possessed by Meseret under SM2. Other alleles are shared by some but not all populations. For instance, allele 209 bp is found in all populations in A1 and SA1 while 460 bp only in all populations in A1 and M1. Only allele of 315 bp is present in populations of all MAEZs. From the entire populations, only Kumato populations meet the assumption of Hardy Weinberg Equilibrium (HWE) (**Table 2**).

Table 2. Diversity indices of LEI0258 locus in Ethiopian indigenous chicken populations based on sampling sites

Pop	N	Na	Ne	I	Ho	He	uHe	F	HWE (p-value)	PAL
Batambe	8	7.000	4.414	1.680	0.750	0.773	0.825	0.030	0.21	
Surta	9	7.000	6.231	1.879	0.667	0.840	0.889	0.206	0.20	315, 385
Amesha_Shinkuri	10	9.000	6.452	2.013	1.000	0.845	0.889	-0.183	0.89	
Gafera	10	8.000	5.556	1.878	0.800	0.820	0.863	0.024	0.2	
025_Adane	10	8.000	5.556	1.861	0.700	0.820	0.863	0.146	0.08	
Meseret	10	12.000	7.692	2.264	1.000	0.870	0.916	-0.149	0.68	185
Hugub	10	7.000	4.000	1.623	0.700	0.750	0.789	0.067	0.09	
Kefis	10	10.000	7.692	2.164	0.700	0.870	0.916	0.195	0.32	
Gesses	10	10.000	5.556	2.011	1.000	0.820	0.863	-0.220	0.93	
Kido	10	10.000	7.407	2.151	0.800	0.865	0.911	0.075	0.15	
Tsion_Teguaz	10	8.000	5.882	1.900	0.800	0.830	0.874	0.036	0.35	
Arabo	10	15.000	11.765	2.597	1.000	0.915	0.963	-0.093	0.71	
Ashuda	10	10.000	7.407	2.151	0.800	0.865	0.911	0.075	0.42	
Dikuli	10	8.000	7.143	2.016	0.800	0.860	0.905	0.070	0.31	
Bekele_Girisa	10	11.000	9.091	2.293	0.900	0.890	0.937	-0.011	0.47	
Shubi_Gemo	10	9.000	5.405	1.942	0.500	0.815	0.858	0.387	0.24	
Kumato	10	9.000	7.143	2.068	0.800	0.860	0.905	0.070	0.02*	277
Loya	10	12.000	10.526	2.415	0.900	0.905	0.953	0.006	0.57	
Hadush_Adi	10	12.000	9.524	2.363	1.000	0.895	0.942	-0.117	0.49	263
Mihiquan	9	12.000	10.125	2.399	0.889	0.901	0.954	0.014	0.33	
Gijet	10	8.000	6.250	1.943	0.700	0.840	0.884	0.167	0.39	
Metkilimat	10	11.000	8.333	2.250	0.900	0.880	0.926	-0.023	0.37	
Alfa_Midir	10	8.000	5.714	1.888	0.800	0.825	0.868	0.030	0.23	
Negassi_Amba	10	6.000	4.167	1.566	0.800	0.760	0.800	-0.053	0.83	
Mean		9.458	7.043	2.055	0.821	0.846	0.892	0.031		
SE		0.438	0.416	0.053	0.026	0.009	0.009	0.027		

*PAL = Private allele; He = Unbiased Expected Heterozygosity/ Nei's Gene diversity = (2N / (2N-1)) * He; I = Shannon's Information Index = -1* Sum (pi * Ln (pi)); *significantly deviate from HWE (P < 0.05); AR = Allelic Richness*

Table 3. Allele frequencies (%) of locus LEI0258 in Ethiopian indigenous chicken populations based on sites.

Allele/n	B	S	A	G	A	M	H	K	GE	KI	TT	AR	AS	DI	BG	SG	KU	LO	HA	MI	GI	MET	AL	NA	P
	8	9	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	9	10	10	10	10	
185	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
197	31	17	0	15	5	0	0	10	35	15	0	15	10	10	15	5	5	10	15	6	10	20	25	20	20
209	0	0	5	0	5	5	10	0	0	0	0	5	0	0	0	5	0	5	5	0	0	0	0	0	8
221	6	0	5	0	25	5	5	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	6
245	0	0	0	0	0	5	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	2
253	6	0	10	5	0	5	0	5	10	10	20	5	5	10	5	10	0	10	5	11	20	15	15	0	19
263	0	0	15	5	5	5	25	0	15	5	0	15	5	10	0	0	10	5	15	6	5	10	5	25	18
277	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	1
289	6	0	5	10	0	0	0	0	10	25	5	5	15	15	0	0	5	10	5	6	10	5	10	0	16
300	0	0	5	0	0	0	0	10	5	0	10	5	0	0	15	35	0	5	10	6	0	0	0	0	10
302	0	0	0	0	0	0	0	5	0	0	0	0	0	0	5	0	10	0	0	0	0	0	0	0	3
312	0	17	0	10	15	15	0	5	5	0	25	5	10	15	10	15	0	0	0	17	15	5	5	10	17
315	31	17	20	30	15	25	10	10	5	10	20	5	25	20	15	5	20	15	15	11	25	15	25	5	24
325	0	0	0	0	0	0	0	5	0	0	0	5	0	0	0	0	0	0	0	6	0	0	0	0	3
327	0	6	0	0	0	0	0	0	0	10	5	0	0	15	0	0	0	10	0	6	10	0	0	0	7
340	0	0	0	0	25	0	5	20	0	5	0	5	0	0	0	0	0	0	5	0	0	0	0	35	7
351	0	0	0	0	0	0	40	10	5	5	0	0	0	0	5	0	0	0	0	11	0	0	0	0	6
363	6	22	25	5	0	15	0	20	5	10	10	10	5	5	5	10	20	10	10	11	0	5	5	0	20
375	13	0	10	20	0	0	0	0	0	0	0	5	0	0	10	0	15	5	5	0	0	10	10	5	11
385	0	0	0	0	0	5	0	0	0	0	0	5	10	0	0	5	0	10	0	6	0	0	0	0	6
397	0	0	0	0	0	5	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	5	0	0	3
411	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	0	0	0	1
426	0	11	0	0	0	0	0	0	5	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3
450	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	5	0	0	2
460	0	0	0	0	5	0	5	0	0	0	0	5	0	0	0	0	5	0	0	0	0	5	0	0	5
465	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	1
472	0	0	0	0	0	0	0	0	0	0	0	5	5	0	0	0	0	0	0	0	0	0	0	0	2
485	0	0	0	0	0	5	0	0	0	0	5	0	0	0	0	0	0	0	0	0	5	0	0	0	3
569	0	11	0	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0	0	0	0	0	0	0	2

BA = Batambe; SU = Surta; AS = Amesha Shinkuri; GA = Gafera; AD = 025-Adane; ME = Meseret; HU = Hugub; KE = Kefis; GE = Gesses; KI = Kido; TT = Tsion Teguaz; AR = Arabo; AS = Ashuda; DI = Dikuli; BG = Bekele Girisa; SG = Shumbi Gemo; KU = Kumato; LO = Loya; HA = Hadushi Adi; MI=Mihiquan; GI = Gijet; MET = Metkilimat; AL = Alifa Midir; NA = Negasi Amba; P = No. of populations sharing the allele

Table 4. Allele frequencies (%) of locus LEI0258 in Ethiopian indigenous chickens across major agro-ecological zones

Allele/n	A1	M1	M2	M3	SA2	SH1	SH2	SM2	P
N	10	10	13	11	10	12	15	13	
185	0	0	0	0	0	0	0	4	1
197	0	5	15	23	5	13	3	12	7
209	10	5	0	0	5	0	0	0	3
221	5	25	0	0	0	0	0	0	2
245	0	0	0	0	0	0	3	4	2
253	0	0	4	14	10	8	0	12	5
263	25	5	4	9	0	8	3	8	7
289	0	0	8	9	0	13	7	4	5
300	0	0	4	0	35	0	7	0	3
302	0	0	0	0	0	0	3	0	1
312	0	15	0	9	15	0	7	8	5
315	10	15	12	23	5	17	20	8	8
325	0	0	4	0	0	0	0	0	1
327	0	0	0	0	0	13	7	4	3
340	5	25	12	0	0	0	0	4	4
351	40	0	0	0	0	4	3	0	3
363	0	0	8	5	10	13	3	4	6
375	0	0	8	9	0	4	13	8	5
385	0	0	4	0	5	0	3	0	3
397	0	0	0	0	0	0	0	8	1
411	0	0	0	0	0	0	10	0	1
426	0	0	12	0	0	8	0	0	2
450	0	0	0	0	0	0	3	4	2
460	5	5	0	0	0	0	0	0	2
465	0	0	0	0	0	0	0	4	1
472	0	0	4	0	0	0	3	0	2
485	0	0	4	0	0	0	0	4	2
525	0	0	0	0	0	0	0	4	1
569	0	0	0	0	10	0	0	0	1

A1 = Tepid to cool arid mid highlands; M1 = Hot to warm moist lowlands; M2 = Tepid to cool moist mid highlands; M3 = Cold to very cold moist sub-afro alpine to afroalpine; SA2 = Tepid to cool semi-arid mid highlands; SH1 = Hot to warm sub-humid lowlands; SH2 = Tepid to cool sub-humid mid highlands; SM2 = Tepid to cool sub-moist mid-highlands; P = N of population sharing the allele.

Allelic sequence polymorphisms

Sequence information including repeat regions and flanking regions are presented at **Table *2*** for a subset of homozygote LEI0258 genotypes. Based on the blastn information from NCBI, there are thirteen new alleles from the 21 alleles sequenced. Variable Number of Tandem Repeats (VNTR) R12 is observed 2 to 17 times but R13 (CTATGTCTTCTTT') only one.

The 23rd to 30th nucleotides downstream of the repeat region are "ATTTTGAG", whilst, 3 alleles sequences were found to have different repeats than respective reference sequences. 24 and 1 SNP substitutions were found at positions 39 and 46, respectively. 12 insertion SNPs and 2 deletions were noted on the upstream polymorphism positions of -30 to -29 positions. Besides, 2 nucleotide substitutions were reported at -61 upstream polymorphism, while, 3 substitutions at -28 position. The consensus sequence size deviation from fragment size ranged from 1 to 115 bp. Further polymorphisms were also observed in different positions of the repeat structure other than the positions considered here under. B10, B11.1, B13, B72 haplotypes were obtained from the allelic sequence. The invariable (monomorphic sites) and variable (polymorphic) sites found were 412 and 35, respectively. A total of 26 singleton sites and 5 parsimony informative sites were observed from the package of DNA sequence polymorphism while the total number of mutation sites and indel events were 33 and 17, respectively.

Discussion

Fragment length based genetic variation in the MHC region

In this study, we report polymorphism at the MHC-B VNTR marker, LEI0258, in indigenous chicken village chicken populations from Ethiopia. From the 29 allele size reported here, 22 are novel alleles (Fulton *et al*., 2006; J. E. Fulton *et al*., 2016; Gupta *et al*., n.d.; Izadi *et al*., 2011; Keambou *et al*., 2014; Lwelamira *et al*., 2008; Ncube *et al*., 2014; Nikbakht *et al*., 2013). The number of alleles reported here is higher than the numbers reported by different authors in previous works on indigenous chicken populations (Han *et al*., 2013; Nikbakht *et al*., 2013). The allele size ranges from 185 and 569 bp with alleles frequencies ranging from 2.5% to 38 % across populations. The maximum allele size reported in this study is larger than the one characterized in other studies (e.g Han *et al*. 2013). Allele sizes of 197, 253, 263, 312, 315 and 340 bp were found in all 24 populations.

The high diversity at the marker may be a direct consequence of the diversity of disease challenges facing Ethiopian chicken within and across different agro-ecologies, with polymorphism at the locus maintained by balancing selection with high LEI0258 diversity increasing the diversity of antigens being presented to T-cells (Chazara *et al*., 2013).

Overall, 50% of the alleles in this study are only found in only two or three of the populations out of the 24 considered. With the exception of alleles, 197, 312, 315 and 351 bp, the remaining of the alleles occurred at lower frequency (< 0.20). The low frequency abundance of LEI0258 alleles might be attributed to a new mutation arising in a population and therefore available only in few individuals. It might also be due to its susceptibility to disease and other selection pressures resulting unfit to survive the production challenge the chicken is facing in the variable environments where they were sampled. High frequency of allele 315 at Batambe population could imply a fitness or survival advantage to the individual carrying it resulting in it being selected for and occurring at higher frequencies (31%). This however needs to be further study in absence of any information regarding the possible association between disease resistance/susceptibility and the allele.

We did not identify here those alleles which previously have been shown to be positive correlation with NDV (206 bp) and body weight (307 bp) (Fulton *et al*., 2006; Fulton *et al*., 2016 and Lwelamira *et al*. 2008). For the later, it may not be surprising considering the small size of Ethiopian village chicken compared to their commercial counterparts; while the former suggests that allele (206 bp) may not be

of relevance to NDV resistance/susceptibility in Ethiopian village chicken. Several LEI0258 alleles were shared among the predetermined populations implying that they have been subjected to either directional selection or due to recombination effect (Nikbakht *et al*., 2013; Salomonsen *et al*., 2014). Alleles 205 and 307 bp, reported in Tanzanian chicken to associate with Newcastle disease antibody response and body weight, respectively, were not reported in Ethiopian chicken in this study (Lwelamira *et al*., 2008). The power marker analysis also showed that the minor allelic frequency of the entire population was 17%.

The mean observed heterozygosity (82.1%) found in this study are far more than the expected level (50%) indicating higher genetic variation in indigenous chicken populations of Ethiopia. In other words, a heterozygous population has a better degree of resisting/tolerating multiple disease infections that challenge chicken populations. From the entire populations, Kumato populations are found to be significantly different from the test of the Hardy Weinberg Equilibrium (HWE). The possible accountable factors that causes this deviation from HWE might be possible introgression with exotic chicken as they are very close to the exotic poultry multiplication center. The other possible reasons might be due to a continuous exposure to disease and parasite challenges (viral/ bacterial infections) that indigenous chicken are exposed to the occurrence of natural selection at MHC locus to combat these challenges. Besides, our sampling might have target related birds.

Allelic sequence polymorphisms and relationships

A subset of allele sequencing result ascertained the presence of single nucleotide polymorphisms (SNPs) in LEI0258. The two main VNTR were the R13 and R12. R13 with a 13 bp repeat unit, "CTATGTCTTCTTT' was found with frequency of only once similar to Wang *et al*. (2014) and inconsistent with other studies with more frequencies (Chazara *et al*., 2013; Nikbakht *et al*., 2013). The 23 to 30 position downstream of the repeat region was sequenced as "ATTTTGAG". They agree with the sequences obtained by Fulton *et al*. (2016), and Han *et al*. (2013). Twenty-four and one SNP substitutions were found at positions 39 and 46, respectively. The number of R12 motifs (CTTTCCTTCTTT) in the individual sequences ranged from 2 to 18, whilst, only one R13 motif was found. 12 insertion SNPs and 2 deletions were noted on the upstream polymorphism positions of -30 to -29 positions. Besides, 2 nucleotide substitution were reported at -61 upstream polymorphism, while, 3 substitution at -28 position. 6 allele sequences were found to have different repeats than respective reference sequences (ATTTTGAG). Results of allele size from both fragment length and consensus sequences did not exactly much which might be because of the difference in environmental factors, technological approaches and precisions. The size deviation ranged from 1 to 115 unlike what was reported by Han *et al*. (2013) who found size differences of 1 to 69 range. The comparison between the fragment sizes and consensus sizes (bp) across population consistently showed higher values for the later except for the Dara population. This result was not consistent with the works of Fulton (2016) and Han *et al*., (2013). Further polymorphisms were also observed in different positions of the repeat structure other than the positions considered here under. Only few haplotypes (B10, B11.1, B13, B72), were found when compared with the haplotypes reported by Chazara *et al* (2013). In contrast, none of these haplotypes were reported in the report of Wang *et al*. (2014) for Chines chicken and Ngeno *et al*. (2015) for Kenyan indigenous chicken.

The SNP based phylogenic analysis using Neighbor Joining (NJ) showed that indigenous chicken populations are mainly clustered into two gene pools comprising different subpopulations as obtained from the structure analysis of allele sizes from capillary electrophoresis. From phylogenetic analysis clear separation of ecotypes were not noted indicating genetic admixture between populations.

Conclusion

Very high diversity was found in Ethiopian indigenous chicken populations at LEI0258, this diversity is observed within all population. Our results support the importance of MHC diversity in response to the disease challenges faced by smallholder poultry production in Ethiopia. Breeding improvement programs will need to maximize this diversity through balancing selection that maintains polymorphisms and increases within population diversity. This very high diversity report for Ethiopian indigenous chicken populations on LEI0258 locus will provide a framework for the existing and future chicken breed improvement interventions. Besides, we can infer that the genotyping of the VNTR marker LEI0258 is a suitable method for MHC typing of indigenous Ethiopian chicken populations considering the high level of polymorphism observed at the locus within and across indigenous populations. Polymorphisms from the sequencing result, also support the genome diversity of indigenous Ethiopian village chicken populations. As a way forward, studying the relationship of these polymorphisms and the disease resistance/susceptibility haplotypes in Ethiopian chicken populations should be undertaken.

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Table 5. Overall polymorphisms identified in the LEI0258 alleles in indigenous chickens of Ethiopia.

[a]Chicken	NCBI Acc. No.	Fragment length (bp, by genotyping)	Consensus size (bp, by sequencing)	Upstream				R13	R12	Downstream					Acc. No.	Haplotype
				Δ	TT	G	TT			C	ATTTGAG	Δ	T	T		
				-61	-30 to -29	-28	-19-18			5	23-30	33	39	46		
Batambe_4H	MG495227	197	193	∘	∘	∘	∘	1	2	∘	Δ	∘	Δ	Δ	DQ239495	B11
Surta_7H*	MG495249	315/426	486	∘	Δ	Δ	∘	1	13	∘	∘	∘	Δ	∘	DQ239562	BW4
Hugub_H2*	MG495230	351/460	236	∘	Δ	∘	∘	1	4	∘	∘	∘	Δ	∘	KF535086	
Hugub_H9*	MG495244	351	345	∘	∘	∘	∘	1	15	∘	∘	∘	Δ	∘	DQ239508	B14
Gafera_8C*	MG495239	315	309	∘	∘	∘	∘	1	12	∘	∘	∘	Δ	∘	KF534941	
Tsion_9C*	MG495231	315/327	250	∘	∘	∘	∘	1	7	∘	∘	∘	Δ	∘	KF534930	
Adane_9C*	MG495245	312/340	357	∘	∘	∘	∘	1	16	∘	∘	∘	Δ	∘	DQ239506	B130
Ngasiamba_4H	MG495243	340	333	∘	∘	∘	∘	1	14	∘	∘	∘	Δ	∘	KF534946	
Ashuda_1C	MG495232	289	283	∘	Δ	Δ	Δ	1	10	∘	∘	∘	Δ	∘	KF535091	
Ashuda_9C*	MG495240	315	309	∘	∘	∘	∘	1	12	∘	∘	∘	Δ	∘	DQ239494	B10
Ashuda_10H*	MG495236	289	295	∘	Δ	∘	∘	1	11	∘	∘	∘	Δ	∘	DQ239550	B72
Dikuli_4H*	MG495237	315/411	307	∘	Δ	Δ	Δ	1	12	∘	∘	∘	Δ	∘	DQ239550	B72
Kefis_12C	MG495233	289	283	∘	Δ	Δ	Δ	1	10	∘	∘	∘	Δ	∘	KF535091	
Dikuli_7H	MG495241	321	321	∘	∘	∘	∘	1	13	∘	∘	∘	Δ	∘	KF534945	
Bekelegirisa_1H*	MG495246	300/302	379	∘	Δ	Δ	Δ	1	18	∘	∘	∘	Δ	∘	KF535100	
Bekelegirisa_8H*	MG495234	312/315	295	∘	Δ	Δ	Δ	1	11	∘	∘	∘	Δ	∘	KF534937	
Shubigemo_1H*	MG495235	300	295	∘	Δ	Δ	Δ	1	11	∘	∘	∘	Δ	∘	DQ239496	B11.1
Kumato_2C	MG495248	197/460	357	∘	Δ	Δ	Δ	1	16	∘	∘	∘	Δ	∘	KF535100	
Kumato_5H	MG495248	302	379	∘	Δ	Δ	Δ	1	18	∘	∘	∘	Δ	∘	KF535100	
Meseret_156b	MG495229	312/315	333	∘	∘	∘	∘	1	4	∘	Δ	∘	Δ	∘	KF534926	
Gijet_49H*	MG495238	253	295	∘	Δ	∘	Δ	1	12	∘	∘	∘	Δ	∘	DQ239550	B72

[a]The codes of chicken populations; [b]Δ Defined deletion compared with the reference sequence. ∘Identical to the reference sequence; * unique alleles

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Goat Production and Marketing System in Arbaminch Zuria and Mirab Abaya Woredas of Gamogofa Zone, Southern Ethiopia

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Abstract

This study was conducted to assess goat production and marketing system, and identify and prioritize production and marketing constraints in Arbaminch Zuria and Mirab Abaya districts of Gamogofa Zone of southern Ethiopia. A total of 120 representative households (2 districts x 2 Agro-ecology x 2 PA x 15 households) were selected for the study following multi-stage stratified sampling. The study employed cross sectional survey with structured questionnaire, focus group discussion and key informants' interview to collect data. According to the respondents, the primary reason for keeping goats in the study area was income generation (index =0.277) followed by saving (index = 0.269). The major feed resources for goats in the study area were browses (road side, communal and private), crop residues, aftermath and improved forages. The two main goat breeding seasons reported by the households were April to June and October to December when feed resources are abundant while goat breeding was totally uncontrolled. Disease and parasites were the major constraints, which caused about 13.7% loss during 12 months' period in Arbaminch Zuria district. Male and female goats achieve age at first service at about 9.4 and 7.9 months, respectively, and their reproductive life is 54.6 and 95.5 months, respectively. Age at first kidding, Kidding interval, Liter size, and weaning age of kids were 13.9, 7.8, 1.24, 4.1 months, respectively. Dominance of brokers, lack of market information and seasonality of price are the major marketing constraints. Introduction and proper implementation of disease and parasite control strategies and market development are suggested to improve offtake rate and thereby enhance income of smallholder producers.

***Key words**: Goats, production system, marketing, reproductive performance.*

Introduction

Ethiopia possesses a large goat population with diverse breed types that are distributed across all parts of the country (Solomon *et al.*, 2014) and home to approximately 32.74 million goats (CSA, 2018) excluding some pastoral areas of Afar and Somali regions. With these resources, the country accounts for 7.2% of African and 2.6% of the global goat population (FAOSTAT, 2015). However, the production and productivity of goats, and the contribution it makes to the national economy is disproportionately low, mainly due to poor nutrition, prevalence of diseases, lack of appropriate breeding strategies and poor understanding of the production system as a whole (Tesfaye, 2009).

On the other side, the demand for goat meat both for domestic and export market has been increasing over time due to increasing income among some segment of the society and health concern. Moreover, seasonal fluctuation of rainfall due to climate change causes losses of food crops at the young stage particularly in lowland and mid altitudes areas of Arbaminch Zuria and Mirab Abaya districts of Gamogofa zone. Keeping small ruminants, particularly goats, is an alternative and preferred strategy to fulfill family needs and generate income in these areas. This is partly because of the physiological

adaptation mechanisms of goats to harsh tropical climate. As a result, goat production has been intensifying in these areas. It is, therefore, important to study production and marketing system of goats and develop context specific recommendations to enhance the production and productivity of goats in the area.

The objectives of this study were, therefore, to assess goat production and marketing system, and identify and prioritize production and marketing constraints in Mirab Abaya and Arbaminch zuria districts of Gamogofa zone, Southern Ethiopia.

Materials and Methods

Description of the Study Area:- The study was conducted in Arbaminch Zuria and Mirab Abaya districts of Gamogofa Zone, Southern Ethiopia. Arbaminch, the capital of Gamogofa zone & Arbaminch Zuria district, is located at 505 km and 275 km south west of Addis Ababa and Hawassa cities, respectively (Fsahatsion et al., 2013). Arbaminch Zuria district is located at an altitude ranging from 1001-2500m.a.s.l. Its latitude and longitude are 6^0N and 37^0E, respectively (Defaru and Tuma, 2013). Agro-ecologically the district is 14% Dega, 53% Woina Dega and 33% Kolla. The Annual temperature ranges from 12.5-27.5^{0}C, while the annual rainfall is between 801-1600mm. Rainfall distribution in the study area is bimodal where long rainy season covers from beginning of March to end of May and a short rainy season from mid-August to mid-October (Shegena *et al*., 2014). The livestock population of the district is 104,642 cattle; 4,616 equines; 35,226 sheep; 20,894 goats and 56,555 chickens (ODLFRD, 2015). Mirab Abaya district is located at 455 km south of Addis Ababa. The district is situated between 1100and 2800m.a.s.l. Its latitude and longitude are 6^0N and 37^0E, respectively (ODLFRD, 2015). The district is divided into three agro-ecological zones, namely, Dega, Woina Dega and Kola which account for about 11%, 27% and 62% of the total area, respectively. The rainfall regime in the district is bimodal. The first round of rain occurs between March and May and the second round of rain occurs from June to August. The district has 46,417 cattle; 2,165 equines; 8,102 sheep; 29,869 goats and 24,071 chickens (Yilikal, 2015).

Sampling techniques: - Arbaminch Zuria and Mirab Abaya districts were purposively selected from the Zone for the study based on potential of high value livestock commodities selected by the Livestock and Irrigation Value Chain for Ethiopian Smallholders (LIVES) project (www.lives-ethiopia.org). From each district, four rural PAs based on goat population, accessibility for transportation and agro-ecology (two midland and two lowland PAs) and 15 households from each PAs comprising 13.3% women were selected for the interview based on their experience and knowledge on goat production and marketing. Thus, the total number of households interviewed was 120.

Questionnaire survey: - Structured questionnaire was used to collect the primary data from the households and summarized version of the questionnaire were used to collect data from FGD and key informants interview. Data were collected by the DAs with a close follow up of the researcher. The secondary data were collected from zonal and district Livestock and Fishery Development Offices and Marketing and Cooperative Offices in zonal and district levels by the researcher.

Data Analysis : - The data collected from the survey were analyzed using Statistical Package for Social Sciences (SPSS ver. 16.0, 2007). Data on percentages and means were analyzed using descriptive statistics and cross tabulation. Indexes were calculated for all ranking data according to the formula: Index = sum of [(5 for rank 1)+(4 for rank 2)+(3 for rank 3)+(2 for rank 4)+(1 for rank 5)] given for an individual reason divided by the sum of [(5 for rank 1)+(4 for rank 2)+(3 for rank 3)+(2 for rank 4

)+(1 for rank 5)] for overall reasons. Treatment means were separated by using the least significant difference and declared significance at p<0.05.

Results and Discussion

Socio-economic characteristic of the households

Household (HH) characteristics: The average age of the household was significantly different (*p<0.05*) between the agro-ecologies of Arbaminch Zuria district except for those (*p>0.05*) in Mirab Abaya district (Table 1). The overall mean total number of family members was consistent across the districts. However, it was higher (*p<0.05*) for households in midland agro-ecology compared to the ones in lowland agro-ecology in both districts. The study showed that about 86.7% of the interviewed households were males while the remaining 13.3% were females across both districts. The average family size was 8.5 in Arbaminch Zuria and 8.3. in Mirab Abaya districts, and these figures were lower than the average family size of 10.5 reported from the Central Rift Valley of Ethiopia (Zewdie and Yoseph, 2014) and higher than the report 6.29 from Ilu Abba Bora Zone (Dhaba *et al.*, 2012). The high family size in the studied districts of the current study area might be due to lack of awareness on family planning and towards the culture of polygamy. Unlike the present findings, the contribution of female headed household was higher in Mada walabu (15%), Sawena (30.8%) and Rayitu (32%) districts of Bale Zone, Oromia region (Belete, 2013). The illiteracy rate of the households in the current study was lower than 67.4%and 65%reportedby Tesfaye (2009) and Tsedeke (2007), respectively and higher than 30% reported from Goma district of Jimma Zone (Belete, 2009).

Table.1 Mean ± SE values of Age, family size, sex and educational status of households in Arba Minch Zuria and Mirab Abaya Woredas

Descriptions	Arba Minch Zuria			Mirab Abaya		
	Midland n=30	Lowland n=30	Average n=60	Midland n=30	Lowland n=30	Average n=60
Age of the HH (years)	47.0±1.38^{a}	42.8±1.20^{b}	44.9±0.95	46.2±1.79	45.9±1.71	46.1±1.23
Total family size	9.20±0.51	7.8±0.52	8.5±0.37	9.0±0.47^{a}	7.5±0.44^{b}	8.3±0.33
Male family size	4.8±0.33	3.9±0.34	4.4±0.24	4.6±0.37^{a}	3.4±0.22^{b}	4.0±0.22
Female family size	4.5±0.29	3.8±0.26	4.1±0.20	4.4±0.27	4.1±0.35	4.3±0.22
Experience in goat farming (years)	15.8±1.21	16±0.96	15.9±0.76	14.8±0.67	15.6±1.05	15.2±0.62
Sex of the HH						
Male	86.7	86.7	86.7	86.7	86.7	86.7
Female	13.3	13.3	13.3	13.3	13.3	13.3
Educational status of the HH						
Illiterate	60	16.7	38.3	76.7	40	58.3
Grade 1-6	30	70	50	13.3	33.3	23.3
Grade 7-12	10	13.3	11.7	10	26.7	18.3

HH= households; n=number of households; SE=standard error; ^{ab} means with the different superscripts across rows are significantly different at (p<0.05)

Land holding and allocation

The overall mean land holdings of the interviewed households are presented in Table 2. The mean total land holding of Arbaminch (1.9 ha) and Mirab Abaya (1.8 ha) districts were consistent. However, the mean landholding per household was significantly higher ($p<0.05$) for lowland compared to midland agro-ecology. Similarly, the mean land holding for crop production was higher ($p<0.05$) for lowland compared to highland agro-climate.

Table 2. Mean land holdings (ha/HH±SE) of sampled stallholders in Arba Minch Zuria and Mirab Abaya woredas

Descriptions	Arbaminch Zuria			Mirab Abaya		
	Midland (n=30)	Lowland (n=30)	Overall (n=60)	Midland (n=30)	Lowland (n=30)	Overall (n=30)
Total land holding of the HH	1.0±0.11^{b}	2.8±0.34^{a}	1.9±0.21	1.0±0.08^{b}	2.6±0.20^{a}	1.8±0.15
Crop land	0.7±0.07^{b}	1.5±0.17^{a}	1.1±0.10	0.7±0.06^{b}	2.0±0.16^{a}	1.3±0.12
Fruit land	0.0±0.0^{b}	0.8±0.13^{a}	0.4±0.08	0.0±0.0^{b}	0.2±0.06^{a}	0.1±0.03
Vegetable land	0.04±0.05	0.12±0.06	0.08±0.03	0.04±0.01^{a}	0.0±0.0^{b}	0.02±0.0
Fallow land	0.06±0.02	0.13±0.05	0.09±0.03	0.08±0.01^{a}	0.0±0.0^{b}	0.04±0.01
Grazing land	0.22±0.04	0.25±0.08	0.24±0.04	0.21±0.02	0.42±0.11	0.32±0.06

n=number of households; SE=standard error; HH=household; ha=hectare; ^{ab} means with the different superscripts across rows are significantly different at ($p<0.05$)

The mean land holding observed in the present study was higher than the overall mean of 1.5±0.07ha reported by the previous study conducted in the highland and midland of Gamogofa Zone (Fsahatsion *et al.,* 2013). However, it was lower than 5.01ha/HH reported for Central Rift Valley of Ethiopia (Felekech *et al.,* 2013). The average crop land holding (1.1±0.10ha/HH for Arbaminch Zuria and 1.3±0.12ha/HH for Mirab Abaya districts) in the present study was higher than 0.94±0.05ha/HH reported for the Zone this study was conducted (Fsahatsion *et al.,* 2013). On the other hand, the mean grazing land holding per household observed in the present study (0.24±0.04ha/HH for Arbaminch Zuria and 0.32±0.06ha/HH for Mirab Abaya districts) was comparable with 0.24±0.02ha/HH reported by Fsahatsion *et al.* (2013) for the highlands and midlands of Gamogofa Zone.

Livestock holdings

The mean livestock species holding of the households in the study area is presented in Table 3. In most instances the mean livestock species holding per household varied significantly ($p<0.05$) between agro-ecologies of both districts. However, there was no significant variation ($p>0.05$) in cattle and castrated yearling goat holding in the midland and lowland of Mirab Abaya district. Similarly, the average household holding of mature castrates, mature females other than does, sheep, mule and chicken was not significantly different ($p>0.05$) for both agro-ecologies of each district.

The overall mean cattle holding (7.1±0.47 for Arbaminch Zuria and 4.8±0.32 for Mirab Abaya districts) in the current study was higher than 3.9±0.29 and 1.7±0.26 reported for Bati and Siti areas of Amhara

and Somali regions, respectively (Hulunim, 2014). However, it was lower than 10.4±1.21 reported by the same author for Borena area of Oromia region.

Table 3. Mean (± SE) livestock holding per households

Livestock Species	Arba Minch Zuria			Mirab Abaya		
	Midland n=30	Lowland n=30	Overall n=60	Midland n=30	Lowland n=30	Overall n=60
Cattle	5.3±0.29b	8.8±0.78a	7.1±0.47	4.3±0.32	5.2±0.54	4.8±0.32
Goats						
· Does	3.3±0.28^{b}	6.9±0.45^{a}	5.1±0.35	3.4±0.31^{b}	6.6±0.62^{a}	5.0±0.40
· Unweaned male kids	1.9±0.23^{b}	4.2±0.35^{a}	3.1±0.25	1.7±0.20^{b}	4.0±0.53^{a}	2.9±0.32
· Unweaned female kids	1.7±0.17^{b}	4.1±0.43^{a}	2.9±0.28	2.3±0.26^{b}	3.6±0.34^{a}	2.9±0.23
· Weaned male kids	0.8±0.14^{b}	2.8±0.24^{a}	1.8±0.19	1.8±0.18^{b}	2.9±0.30^{a}	2.3±0.18
· Weaned female kids	1.3±0.14^{b}	3.5±0.29^{a}	2.4±0.21	1.7±0.22^{b}	2.6±0.32^{a}	2.2±0.20
· Castrated yearlings	0.4±0.09^{b}	0.9±0.15^{a}	0.7±0.09	0.13±0.08	0.20±0.09	0.2±0.06
· Non-castrated yearlings	0.6±0.14^{b}	2.2±0.29^{a}	1.4±0.19	1.2±0.14^{b}	2.8±0.32^{a}	1.9±0.20
· Yearling females	0.7±0.19^{b}	2.7±0.37^{a}	1.7±0.24	0.8±0.16^{b}	2.9±0.36^{a}	1.8±0.24
· Mature castrates	0.2±0.11	0.9±0.21	0.6±0.13	0.4±0.10	0.4±0.13	0.4±0.08
· Mature non-castrates	0.5±0.10^{b}	1.3±0.23^{a}	0.9±0.14	0.9±0.09^{b}	2.0±0.25^{a}	1.4±0.15
· Mature females other than does	0.9±0.16	1.9±0.36	1.4±0.21	1.3±0.16	2.0±0.36	1.7±0.20
· Total goats	12.3±1.75^{b}	31.4±3.37^{a}	22±2.28	15.6 ±1.9^{b}	30±3.62^{a}	22.7 ±2.26
Sheep	1.5±0.42	0.7±0.55	1.1±0.34	0.10±0.10	0.0±0.0	0.05±0.05
Mule	0.1±0.06	0.0±0.0	0.05±0.03	0.03±0.03	0.0±0.0	0.02±0.02
Donkey	0.4±0.09^{b}	0.7±0.12^{a}	0.6±0.08	0.8±0.08^{a}	0.2±0.07^{b}	0.5±0.06
Livestock other than chicken	19.6±2.61^{b}	41.6±4.82^{a}	30.8±3.2	20.8±2.43^{b}	35.4±4.23^{a}	28.1±2.71
Chicken	10.3±0.77	9.9±1.23	10.1±0.72	8.1±0.80	9.2±1.35	8.6±0.78

n=number of households; SE=standard error; ^{ab} means with the different superscripts across rows are significantly different at (p<0.05)

Purpose of goat farming

The ranked result of purpose of goat keeping as by the respondents is presented in Table 4. Accordingly, income generation was ranked as the primary reason for keeping goats with the highest index values of 0.277 followed by saving. This was consistent across the districts and agro-ecology involved in this study. The finding was in agreement with the report from Ilu Abba Bora Zone (Dhaba *et al.*, 2012) and from Uganda (Charles *et al.*, 2015).

Table 4 Overall ranks of purpose of keeping goats

Parameters	Ranks given by the households					
	1st	2nd	3rd	4th	5th	Index
Source of income	49	42	28	1	-	0.277
Saving	41	48	26	5	-	0.269
Meat consumption	-	-	11	62	47	0.114
Use of manure	8	14	43	39	16	0.177
Social capital	22	16	12	13	57	0.163

Goat production system in the study area

Feed resources, feeding and its seasonal availability

Although, communal grazing land is the common feed source for goats and other livestock in the study area, road side browsing was ranked first based on average rank result of both agro-ecologies followed by communal browsing lands and private browsing land (Table 5). Despite, difference in priorities, similar feed resources were reported from Metema district of North Gonder Zone (Tesfaye, 2008) and Rayitu district of Bale Zone (Teshome, 2006).

The major feed resources prioritized in the study area were covered mainly by shrubs and indigenous browse species which are very crucial during dry season.

Table 5 Major feed resources for goats as ranked by the households

Feed resources	Ranks						Index
	1st	2nd	3rd	4th	5th	6th	
Private browsing land	29	40	46	5	-	-	0.227
Road side browsing	43	53	24	-	-	-	0.246
Communal browsing land	47	21	35	16	1	-	0.229
Crop residue	-	4	1	64	31	20	0.118
Improved forages	-	-	-	-	42	78	0.064
Aftermath grazing	1	2	14	35	46	22	0.116

Seasonal availability of different feed resources used for goats in the study area is presented in Table 6. Accordingly, the major feed resource available during the dry season were browses from private and communal areas, crop residues and grazing on crop aftermath in midland while it was browsing from communal area in lowland agro-climate. On the other hand, the dominant feed resources for goats during the rainy season across both agro-ecology were browses from private, communal and roadside areas. Consistent with the current study, seasonality in availability of feed resources for livestock was also reported from Burie district of West Gojjam Zone (Yenesew, 2010) and Metema district of North Gondar Zone (Tesfaye, 2008).

Table 6 Seasonal availability of major feed resources for goats

Feed resources	Mid-land			Low-land		
	Sept-Dec	Jan-Mar	Apr-Aug	Sept-Dec	Jan-Mar	Apr-Aug
Private browsing land	+++	++	+++	+++	+	+++
Road side browsing	+++	+	+++	+++	+	+++
Communal browsing	+++	++	+++	+++	++	+++
Crop residue	+++	++	-	+++	+	++
Improved forages	+	-	+	+	-	+
Aftermath grazing	++	++	-	+	-	+

+++= good feed availability; ++=moderately available; +=less available; - =not available.

Source of water for goats in the study area

The major source of water for goats in the study area included rivers, wells, springs, lakes (Lake Abaya) and tap water (Table 7). The highest (40%) proportion of annual water for goats comes from river. The other source of water for goats and other livestock in the selected lowland PAs of Mirab Abaya district was tap water and which contributes 22.5% of the total annual water required for goats. According to FGD, this was constructed 22 years ago by a non-governmental organization. The major water sources founded in the current study were in line with the report from Dale district of Sidama Zone (Endeshaw, 2007). Similarly, river water as a major source of water for goats was reported from Ebnat and Farta districts of Amhara region (Damitie *et al.*, 2015). In corroborate with the current study, Lakes and rivers were one of the major water sources for goats in Central Rift Valley of Ethiopia (Felekech *et al.*, 2013). Frequency of watering is twice a day, early morning when the animals are moving for browsing and returning back from browsing in the late afternoon.

Table 7. Sources of water for goats

Source of water	Agro-ecology		Total n (%)
	Midland n (%)	Lowland n (%)	
River water	28 (46.7)	20 (33.3)	48 (40)
Well water	6 (10)	3 (5)	9 (7.5)
Spring water	26 (43.3)	-	26 (21.7)
Lake Abaya	-	10 (16.7)	10 (8.3)
Tap water	-	27 (45)	27 (22.5)

n= number of households interviewed

Housing of goats

Goat keepers in the study area confine all sex and age groups of goats together including kids. Providing shelter in the adjoining to the family house is predominant in the midlands (60%) and confining goats in purposely constructed separate houses (88.3%) is predominant in the lowlands (Table 8). In the

current study about 36% and 35% of the households in Burie district of West Gojjam provide separate and adjoining houses, respectively (Yenesew, 2010). Contrary to the current study, the highest proportions (72%) of the interviewed households in the same study used corrugated iron sheet for the roof of goat houses. Provision of house for goats was also reported by Belete *et al.*, (2015) from Bale Zone where the dominant housing system was kraal (45.6%) followed by separate house (28.10%) and yards (25.3%).

Table 8 Types of goat houses reported by the respondents

Type of house	Agro-ecology		Total
	Midland	Lowland	
	n (%)	n (%)	n (%)
Adjoining house	36(60)	-	36(30)
Separate housing	24(40)	53(88.3)	77(64.2)
No house or around the main house	-	7(11.7)	7(5.8)

n= number of interviewed households

Methods of castration and culling of goats

From the total households interviewed, about 51.7%and 48.3% castrate their goats in traditional and modern method, respectively. The result is comparable with the reports from Bati, Borena and Siti area of Amhara, Oromia and Somali regions, respectively (Hulunim, 2014), where more than 50% of the respondents castrates their bucks traditionally. On the other hand, higher proportion (91%) of respondents had access to modern castration services in Ilu Abba Bora Zone (Dhaba *et al.*, 2012).

The wrong perception of farmers to modern castration that says it would delay fattening period caused some farmers to prefer traditional castration. Lack of access to modern castration also forces some midland producers to use traditional castration methods. Generally, all households practice castration to fetch more money from the sale of fattened goats. The observation was in agreement with the previous studies from different parts of the county (Belete, 2009; Takele *et al.*, 2006).

The major reasons of culling goats in the study area are given in Table 9. Breeding females with poor mothering ability, that fail to conceive after repeated mating and abort due to unknown reasons were primarily culled from the flock with an index 0.297 followed by physical damage with an index 0.219. The primary reason for culling goats in the present study area was in agreement with the previous report for Daro-Labu district of West Hararghe (Dereje *et al.*, 2013).

Table 9 Major reasons for culling goats in the study area

Culling reasons	Ranks					Index
	1st	2nd	3rd	4th	5th	
Kidding problem	74	24	22	-	-	0.297
Physical defect	7	26	62	25	16	0.219
Unwanted physical characteristics	28	35	5	18	13	0.192
Sickness	-	-	1	31	88	0.086
Oldage	12	30	30	48	3	0.206

Index = sum of [(5 for rank 1)+(4 for rank 2)+(3 for rank 3)+(2 for rank 4)+(1 for rank 5)] given for an individual reason divided by the sum of [(5 for rank 1)+(4 for rank 2)+(3 for rank 3)+(2 for rank 4)+(1 for rank 5)] for overall reasons.

Goat health management

According to cross sectional survey and FGD results, the most common diseases and parasites that hamper goat production and productivity in the study area included Tuberculosis, Trypanosomiasis, Coccidiosis, foot rot, pink eye, sheep and goat pox, PPR and CCPP, Ovine pasteurellosis, internal parasite (tape worm), external parasites such as ticks and mites. According to the survey, FGD and key informants interview results; disease occurrence was higher in the months from January to April in the study area.

The reported mean death and mortality by the households over the last 12 months of this study is given in Table 10. From the households interviewed, about 65% in midland, 48.3% in lowland and overall 56.7% reported that they have lost one to three goats due to different health problems in the past 12 months of the present study.

Overall, higher mean mortality of 8.83%, 13.7%, 20.98% and 9.82% was reported for unweaned male kids, unweaned female kids, weaned male kids and weaned female kids, respectively from Arbaminch Zuria district whereas the corresponding values of 13%, 8.3%, 4.5% and 6.76%, respectively were recorded in Mirab Abaya district. The pre-weaning death of 2.1% male and 2.5% for females in the lowland and also 0.6% for male and 1.5% for females in the midlands reported from the study conducted in Daro-Labu district of West Hararghe, Eastern Ethiopia (Dereje *et al.*, 2013) was very lower than the mortality reported in the current study for the same age groups in both studied altitudes of both districts.

The average mortality of unweaned female kids and weaned male kids in Arbaminch zuria and also unweaned male kids in Mirab Abaya district of the current study was higher than 12% reported for kids in the highlands of Ethiopia (Gebremedhin *et al.*, 2015). Mean mortality of unweaned male kids in Arbaminch zuria and unweaned female kids in Mirab Abaya districts of the current study area was lower than 11.5% pre-weaning mortality reported for Somali goat kids under station management at Haramaya University (Zeleke, 2007). The average mortality of does in the present study area was lower than 9.7% reported from the highland of Ethiopia (Gebremedhin *et al.*, 2015).

Reproductive performance of goats

The reproductive performances reported by the households in the current study were significantly varied ($p<0.05$) between studied agro-ecologies in both districts and which were mostly better for lowlands as compared to midland agro-climates (Table 11).

The mean age at first service (AFS) of male goats in the current study was higher than 7.4 months reported for Metema district (Tesfaye, 2009) and 6.6 months reported for Alaba district (Tsedeke, 2007) but lower than 12.3 months reported for Abergelle goats from Metema district (Solomon, 2014).

Age at first kidding (AFK) of goats in the present study was in line with 13.75 months reported for goats from coastal regions of Bangladesh (Rume *et al.*, 2011), but higher than 11.9 months reported for indigenous goats in Alaba district of southern Ethiopia (Deribe, 2009) and lower than 16.7 months reported for Lare and Jikawo districts of Gambella region, South-Western part of Ethiopia (Tsigabu, 2015).

Table 10. Mean death and mortality (%)/HH of goats in the last 12 months of this study

Classes of goats	Arba minch						*Mirab Abaya*					
	Midland		Lowland		Average		Midland		Lowland		Average	
	Death	M (%)	Death	M (%)	Death	M (%)	Death	M (%)	Death	M (%)	Death	M (%)
Does	0.13	3.94	0.33	4.78	0.23	4.36	0.30	8.82	0.20	3.03	0.25	5.93
Un weaned male kids	0.20	10.53	0.30	7.14	0.25	8.83	0.40	23.53	0.10	2.5	0.25	13
Unweaned female kids	0.37	21.76	0.23	5.61	0.30	13.7	0.17	7.39	0.33	9.2	0.25	8.3
Weaned male kids	0.27	33.75	0.23	8.21	0.25	20.98	0.10	5.55	0.10	3.45	0.10	4.5
Weaned female kids	0.17	13.07	0.23	6.57	0.20	9.82	0.23	13.53	-	-	0.12	6.76
Castrated yearling	-	-	0.03	3.34	0.02	1.67	-	-	-	-	-	-
Non castrated yearling	0.07	11.67	0.13	5.91	0.10	8.79	0.07	5.83	0.07	2.5	0.07	4.2
Mature castrates	-	-	-	-	-	-	0.03	7.5	-	-	0.02	3.75
Mature non castrates	-	-	0.10	7.69	0.05	3.84	0.07	7.78	-	-	0.03	3.89

M=Mortality

Kidding interval (KI) reported in the present study was in line with 7.9 months reported for goats in Goma district of Oromia region (Belete, 2009). The mean kidding interval of goats in the current study was higher than 6.3 months reported for western lowland goats of Amhara region (Solomon, 2014) and 5.5 and 6.6 months reported for Woito guji and Central highland goats, respectively (Netsanet *et al.*, 2016). However, it is lower than 10.5 months reported for Saanen goats raised in Sudan (Safaa *et al.*, 2015).

Average litter size of goats in the present study was similar with the report from central highland of Ethiopia (FARM-Africa 1996). Moreover, the mean litter size reported in the current study was higher than 1.04 reported for Abergelle goats (Deribe, 2008) and 1.06 reported for Black Bengal goats managed in semi-intensive system in Bangladesh (Faruque *et al.*, 2010). However, the mean litter size reported in the current study was lower than 2.64 reported for goats in selected coastal regions of Bangladesh (Rume *et al.*, 2011).

Table 11 Mean (±S.E.) reproductive performances of goats as reported by the households

Descriptions	Arba Minch			Mirab Abaya		
	Midland	Lowland	Overall	Midland	Lowland	Overall
	n=30	n=30	n=60	n=30	n=30	n=60
AFS in male goat (m)	10.7±0.29^{a}	8.10±0.29^{b}	9.40±0.27	10.0±0.33^{a}	8.5±0.28^{b}	9.3±0.24
AFS in female goat(m)	9.20±0.35^{a}	6.90±0.13^{b}	8.0±0.24	8.50±0.28^{a}	7.20±0.19^{b}	7.8±0.19
Age at first kidding(m)	15.2±0.34^{a}	12.9±0.13^{b}	14.0±0.24	14.5±0.28^{a}	13.4±0.25^{b}	13.9±0.19
Kidding interval(m)	8.30±0.08^{a}	7.50±0.08^{b}	7.90±0.08	8.30±0.11^{a}	7.40±0.10^{b}	7.80±0.09
Average litter size (N)	1.13±0.02^{b}	1.36±0.02^{a}	1.24±0.02	1.10±0.02^{b}	1.34±0.02^{a}	1.23±0.02
Natural weaning age(m)	4.60±0.09^{a}	3.40±0.10^{b}	4.20±0.08	4.50±0.08^{a}	3.60±0.08^{b}	4.10±0.08
Life time kid production (N)	13.5±0.31^{b}	15.3±0.27^{a}	14.4±0.23	13.9±0.33^{b}	15.4±0.19^{a}	14.6±0.21
RP life span of male goat(m)	54.4±1.11	55.0±1.04	54.7±0.75	54.4±1.38	54.6±1.09	54.5±0.87
RP life span of female goat (m)	96.8±2.07	92.1±1.24	94.4±1.23	100±1.53^{a}	93.2±0.98^{b}	96.6±1.01

n=number of households; m=months; N=number; SE=standard error; AFS=age at first service; RP=reproductive;$^{a\ b}$ means with the different superscripts across rows are significantly different at ($p<0.05$)

Life time kid production reported in the current study is comparable with the 13.5 reported for goats in Metema district of Amhara region (Tesfaye, 2009), but higher than 11 reported for goats in pastoral and agro-pastoral production systems of Southern Ethiopia (Adugna and Aster, 2007). However, the life time number of kid production in the current study was lower than 17.3 reported for western lowland goats (Solomon, 2014).

Weaning age of kids reported in this study was significantly lower ($p<0.001$) for goats in lowland agro-ecology compared to midland. The overall mean weaning age (4.1 months) of goats reported in the current study was in agreement with 4 months reported for goats in Metema district of Amhara region (Tesfaye, 2009). However, it was lower than 5.5 months reported for central highland and Woito guji goats (Netsanet *et al.*, 2016).

Marketing of goats in the study area

Mode of marketing and price setting: - Due to absence of standardized marketing system, producers have less power in deciding price.The dominant (95% of the midland and 31.7% of the lowland) respondents reported that they sale their goats by eye ball price estimation. The remaining households' sale their goat by both eye ball price estimation and live weight basis based on access to mode of marketing in the market. Goat price setting by eye ball estimation wastraditional method of marketing and also reported from other parts of the country (Hulunim, 2014; Belete, 2009; Endeshaw, 2007).

Goat marketing channels and routes

According key informants interview and physical observation of the market, five main marketing channels were identified in the study area as listed below.

Channel 1: Producer => Consumer. Thus, consumers in the local area purchase goats directly from the producers for various purposes.

Channel 2: Producer=>Butcher=Consumer: This marketing channel connects producer with consumer through butchers. The butchers in the towns of the district and Zone purchase animals directly from producers and supply meat to consumers after value addition.

Channel 3: Producer =>Small traders =>Butcher =>Consumers: In this channel, all small traders buys goats from producers at local markets in the rural areas and trek to district towns and Zonal City to sell to butchers, which slaughter and sell meat to consumers.

Channel 4: Producer =>Small trader =>Medium trader =>Big traders=> abattoirs and live animal exporters: In this marketing channel, small traders purchase goats from producers at local market and trekto secondary market and sold to medium traders. Then after, medium traders' transports goats to Humbo (terminal) market in Wolaita Zone, which borders the study districts in the Northern direction. Humbo is 105 and 45 Km far from Arbaminch Zuria and Mirab Abaya districts towns, respectively. At Humbo, medium traders sell goats to big traders who latter on transports the animals to export abattoirs and live animal exporters located in Modjo, Adama (Nazreth) and Addis Ababa. Consumers and butcher houses in Humbo town & Sodo City also purchases animals from medium traders in Humbo market.

Channel 5: Producer =>Medium trader =>Big trader =>Consumer: In this marketing channel, producers' sales goats to medium traders in the medium (secondary) market. Then after, medium trader transports goats to Humbo (terminal) market and sale to bigger traders as described above. Unlike channel 4 which connects producers with medium traders through small traders, this channel connects producers with medium traders directly and thereby makes the channel shorter.

Constraints of goat production and marketing

Constraints of goat production

The major constraints of goat production as ranked by the sampled households in the study area are given in Table 12. Accordingly, disease and parasites are the most limiting constraints of goat production (index=0.235) in the study area followed by predators (index= 0.224) which attacks goats at the night and on the browsing area during the day time. According to focus group discussants, the major predators that attack goats in the study area included fox, monkey, leopard/tiger and hyena.

Table 12 Major constraints of goat production as ranked by the households

Parameters	Ranks							Index
	1st	2nd	3rd	4th	5th	6th	7th	
Predators	45	62	9	4	0	0	0	0.224
Disease and parasites	68	47	5	0	0	0	0	0.235
Labor shortage	5	2	16	47	28	24	0	0.134
Shrinkage of browsing land	0	0	0	15	39	24	42	0.080
Inadequate extension support	0	0	62	14	10	18	16	0.135
Lack of animal health professionals	2	4	27	34	19	29	0	0.127
Lack of veterinary institutions	0	0	1	6	24	27	62	0.065

Index = sum of [(7 for rank 1)+(6 for rank 2)+(5 for rank 3)+(4 for rank 4)+(3 for rank 5)+(2 for rank 6)+(1 for rank 7)] given for an individual reason divided by the sum of [(7 for rank 1)+(6 for rank 2)+(5 for rank 3)+(4 for rank 4)+(3 for rank 5)+(2 for rank 6)+(1 for rank 7)] for overall reasons.

The major production constraints reported by the households in this study were in accordance with the reports from Metema district (Tesfaye, 2009; Solomon, 2007). Moreover, consistent with the current study, high prevalence of disease and parasites were the most important factors limiting goat production in Metema and Abergelle districts of the Amhara Region (Solomon, 2014).

Constraints of goat marketing

The major constraints limiting goat marketing as ranked by the sampled household in the study area is presented in Table 13. Thus, dominance of broker, lack of market information, seasonal fluctuation of market price and lack of transportation were ranked from 1st to 4thwith an index of 0.305, 0.289,0.236 and 0.170, respectively. The reported goat marketing constraints in the current study were in agreement with Getachew *et al.* (2008). Brokers also lower the price of animal during bargaining with producers because of the fact they collect better commission from medium traders. The finding was in line with the report from western Ethiopia (Yilma *et al.*, 2015).

Generally, there was no public market information source for goat value chain actors in the study area. It is believed that absence of market information exposes smallholder producers for exploitation by brokers. The report was in consonance with one of the main small ruminant (sheep) marketing constraint in Burie district (Yenesew *et al.*, 2013)

Table 13 Major constraints of goat marketing as ranked by sampled households

Marketing constraints	Ranks				Index
	1st	2nd	3rd	4th	
Lack of market information	37	37	42	4	0.289
Dominance of brokers	43	47	23	7	0.305
Seasonal fluctuation of market price	31	21	28	40	0.236
Lack of transportation	9	15	27	69	0.170

Index = sum of [(4 for rank 1)+(3 for rank 2)+(2 for rank 3)+(1 for rank 4)] given for an individual reason divided by the sum of [(4 for rank 1)+(3 for rank 2)+(2 for rank 3)+(1 for rank 4)] for overall reasons.

Conclusions

Goat farming is an important component of livestock production in Ethiopia. Accordingly, goats are mainly kept for income generation followed by saving, manure, social capital and meat consumption in the study area. The major feed resources for goats in the study area were browses (road side, communal and private), crop residues, aftermath and improved forages. The two main goat breeding seasons reported by the households were April to June and October to December when feed resources are abundant while goat breeding was totally uncontrolled. The reproductive performances of goats in the current study were higher for goats in lowland agro-ecology compared to midland across both districts. Disease and parasites are the most limiting constraint of goat production in the study area. Dominance of brokers, lack of market information and seasonality of price are the major marketing constraints. Involvement of institutions for the development of the sector is very low. Most of the browses species are tolerant to draught, contain high protein and better support ruminant production during the dry season, Thus, the comparative advantage of goat farming over crop production should be looked in these areas, and effort should be made to conserve and sustainably use these resources. Introduction and proper implementation of disease and parasite control strategies and market development are suggested to improve offtake rate and thereby enhance income of smallholder producers. future strategy should focus on improving the production, productivity and income of livestock producers following the value chain framework and market oriented extension approach.

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Analysis of Eating Quality in Sensory Panelist and Instrumental Tenderness of Beef from three Cattle Breeds in Oromia, Ethiopia

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Abstract

Meat is one of the most nutritious animal products that humans can consume, particularly in terms of supplying high-quality protein, minerals and essential vitamins. Hence, the demand for meat is not only quantity wise, but also quality wise. The objective of this study was to evaluate eating qualities of beef produced at public abattoirs from Arsi, Bale and Harar cattle breeds with semi-trained sensory panel and instrumental tenderness. The samples were collected from the longissimus dorsi region between 12th and 13th ribs within 45 min after slaughters. The samples were packaged in vacuum-seal and aged for14 days to evaluate instrumental tenderness using Warner Bratzler Shear Force Device and eating quality using panel testing. Mean values of 33.12 N, 7.12, 7.2 and 7.24 were determined in instrumental tenderness, sensory tenderness, juiciness, and flavor of beef, respectively. The parameters were significantly affected by age, breeds and season interactions (P<0.01). Breed interaction with age and season exhibited significant variation (P<0.05) on water holding capacity. Beef pH was significant affected by season. The instrumental tenderness had negative medium relationship with sensory attributes conducted for tenderness (r=-0.48) and juiciness (r=-0.27), but positive with flavor (r = 0.60). From this study, it was concluded that quality of beef produced in study areas was relatively tender which internationally competent but becomes tough as cattle gets older. It is recommended that strategy should be developed to encourage premium payment for young cattle marketing that is not exposed to draft service and creating awareness among stakeholders on quality beef production.

***Keyword**:* Beef, Breeds, Ethiopia, Instrumental tenderness, Sensory panel testing

Introduction

Meat is one of the most nutritious animal products that humans can consume, particularly in terms of supplying high-quality protein (essential amino acids), minerals (iron) and essential vitamins (Gebeyehu *et al*., 2013). However, the annual meat per capita consumption in Ethiopia is 8.5 to 10 kg/year, which is very low compared to the average value of Sub-Saharan Africa and the potential expected (EDMIDI, 2016). In contrast, the demand for meat is increasing not only quantity wise but also quality wise primarily based eating quality (Lee *et al*., 2012). However, eating quality of meat are affected by nutrition and management, slaughter age, season, breed, live weight, sex, pre- and postslaughter handling (Purwin *et al*., 2016). Particularly, tenderness has been linked to animal's age, marbling, muscle location and aging (Farzad, 2014). The older animals are the tougher meat source in general (Berit, 2012).

The breed of animals for slaughter is one of the key factors determining the quality of meat and hence, different cattle breeds have different demand for international and domestic beef consumption.

Knowing the effect of age season on eating quality of meat is essential for proper management, improvement and utilization and market promotion. Seasonal variation affects the quality of beef cattle due to feed resources availability is closely related with season of year (Knee *et al*., 2004). Diet is the major factor influenced beef quality like sensory, physical and proximate composition). Finishing diets changed ruminal biohydrogenation of polyunsaturated fatty acids without affecting the concentrations of conjugated linoleic acid in the meat (Dzinic *et al*., 2015)

Animals can be stressed by improper pre-slaughter handling during transport and stunning that could result in undesirable pH of carcass, which causes for pale soft exudative (PSE) and dark firm dry (DFD) meat, poor water-holding capacity and end up in poor cooking loss (Adzitey and Nurul, 2011). The DFD meat has a high ultimate pH, which exposes meat for high microbial contamination (Węglarz, 2012). Quality of meat can be analyzed through both instrumental and panel testing (AMSA, 2015). Information on eating quality of beef produced evaluated using sensory panel testing and instrumental means is inadequate in Ethiopia. This study was therefore, designed to investigate eating quality of beef produced at public abattoirs using trained sensory panelists and instrumental tenderness methods to evaluate PSE, DFD and water holding capacity (WHC) of beef from different breeds and ages of cattle slaughtered for local market.

Materials and Methods

Meat samples collection and aging

In total 118 samples were collected during the dry (October to early November 2017) and wet (mid to end June 2018) seasons. Daily slaughtered cattle were divided into age groups and breeds. Breeds (cattle types) were identified using the phenotypic traits in combinations of coat color, confirmation, dewlap structure, ear, horn and hump type according to Rege and Tawah (1999) and DAGRIS (2011). The samples were collected from three breeds. The *Arsi* and *Bale* breeds were collected from Adama city municipal abattoir while the samples from Harar breeds were collected from Haramaya University. Age of cattle was determined using dentition according to Verification Guidelines (Torrell, 1998). For the purpose of this study the age was stratified into four groups < 5, 5-7, 7-9, and > 9 years old. The sample source cattle were tagged in the abattoir using simple random sampling technique and collected from specific location of longissimus-*dorsi (LD)* muscle between 12^{th} and 13^{th} ribs in abattoirs. Then, collected samples were packed into plastic bags, sealed into vacuum packed, stored in the icebox and aged in deep freeze for 14 days

Screening and training sensory panelists

Panelist screening: The panelists were selected from Oda Bultum University (OBU) 3^{rd} year Dairy and Meat Technology (DMT) students based on their interest and ability to understand the scales rate. Based on the criteria, twelve panelists were selected and participated on the panel test.

Steak preparation and sensory panelist testing: An aged samples were thawed for 24 hr and prepared for both sensory and instrumental tenderness evaluation. The steak preparation was done according to Warner-Bratzler Shear Force (WBSF) procedures protocol developed by AMSA (2015).

$$\text{Cooking loss calculate (CL)} = \frac{\text{(Initial weight – final weight)}}{\text{Initial weight}} * 100$$

Sample presentation: Similar sample presentation procedures were conducted for all panelists at room temperature (24-25 ^{o}C). The steaks were cut uniformly to reduce any bias related to serving position and presented in white color plate randomly. Each Panelist has evaluated three most important eating quality (tenderness, juiciness, and flavor) parameters. Panelists scored each sample on a **9**-point hedonic scale. Between each sample tasted, biscuit and clean water were served for calibration of the panelist taste (AMSA, 2015).

Determination of instrumental tenderness: The prepared steak was cooled at room temperature for one hour prior to determine tenderness by WBSF apparatus as follows: connective tissue across the long axis of the steak was removed to expose the fiber direction. Six cores of 1.27 cm diameter were removed from each sampl**e** parallel with the muscle fibers. The shear force was measured across the middle (center) on each core using WBSF value and expressed in Newtons (N).

Determination of Water Holding Capacity (WHC): Water-holding capacity of meat was measured using the gravimetric method. The fat part of the sample was removed and weighed before the hanging, then reweighed after stored for 24 hrs in a deep freezer (Honikel, 1998). The WHC expressed, in percentage from a total of meat water contents (75%) was calculated as follows;

$$\text{Water holding capacity (WHC)} = \frac{\text{Initial weight of sample - Weight after 24hrs}}{\text{Initial weight}} * 75$$

Determination of pH in Meat: The initial and final pH of meat samples were measured after slaughter at 45 min and 24 h, respectively using digital pH meter. The pH meter probe was calibrated by inserting inserted into distilled water and a buffer solution, then touch a probe with meat and read the value of pH after about 30 seconds (ESVLDM, 2005).

Statistical Analysis: General leaner model procedure of SAS (version 9.0) software was used for analysis of data collected. Where significance difference between fixed effects was observed mean separation was done by DMRT at $P \leq 0.05$. The model used was; $Y_{ijfk} = \mu + \alpha_i + \beta_j + C_f + \gamma_k + e_{ijfk}$

Where; Y_{ijfk} = the response variable

μ = Overall mean common to all observation (Ai, βf, Cf)

α_i = Effects of breed or cattle type (Harar, Arsi and Bale)

β_j = Effect of age (<5, 5-7, 7-9, > 9),

C_f= Effects of season (Dry and Wet)

γ_k = Interaction effect (Breed, Season and Age)

e_{ijfk} = Random error

Results and Discussion

Effect of season

Mean value of eating quality, WHC, pH, and CL of beef samples are presented in Table 1. The overall mean value of sensory panelists and WBSF (N) rated 7.29, 7.16, 7.35, 42.94 N in the wet and 7.12, 7.23, 7.13 and 23.3 N in the dry season for sensory tenderness, juiciness, flavor and instrumental tenderness of studied beef cattle, respectively. The mean value of WBSF for all beef cattle breeds evaluated in this study was 33.12 N, of which 58.47% were tender, 28.81% were tough and 12.71%

were moderate beef, based on the categories described by Calkines and Sulivan (2006) who grouped beef muscles into three tenderness groups. These were instrumental tenderness <37.31 N tender, 37.49 – 44.54 N intermediate and > 44.98 N as tough meat. The present result is comparable with Giusti *et al*. (2013) who reported that shear force values for the Canchim (37.71 N) and Nellore (41.67 N) beef animals after seven days of aging in Brazil.

Table 1. Overall mean value of eating quality, WHC, pH, and CL of three beef cattle as affected by wet and dry seasons in Ethiopia

Variable	Wet season (N= 70)				Dry season (N =48)				Overall Mean
	Min	Max	Mean	SD	Min	Max	Mean	SD	
WBSF (N)	20.57	75.38	42.94	13.82	8.66	47.33	23.3	11.30	33.12
Tenderness	5.57	8.50	7.29	0.63	5.20	8.10	7.12	0.57	7.21
Juiciness	5.78	8.00	7.16	0.51	5.87	8.10	7.23	0.53	7.20
Flavor	6.20	8.45	7.35	0.48	5.50	8.30	7.13	0.78	7.24
WHC	67.07	74.37	71.95	1.56	69.30	74.37	73.82	1.43	72.89
CL	11.96	33.95	21.07	5.70	2.42	22.73	9.99	5.17	15.53
Initial pH	5.90	6.96	6.55	0.23	6.00	7.10	6.50	0.26	6.52
Final pH (24 hrs	5.24	6.20	5.81	0.24	5.30	6.40	5.62	0.23	5.72

WBSF= Warner Bratzler Shear Force in Newton, WHC= Water Holding Capacity

The mean values of instrumental tenderness indicated that there was enormous difference between dry 23.3 N (tender) and wet season 42.94 N (intermediate). This might be due to good conformation of cattle during the dry season because of good quality feed resource availability, long time rest of draft cattle without physical work (traction), and less cold and hot weather stress. Farmer in mixed crop livestock system feed their bulls for sale on at the onset of dry season. In contrast to the general fact that cattle fatten well in wet season, particularly, short rainy, cattle were in poor body condition during this time in the study areas. During the short rainy season from March to June, cattle had passed sever long dry season (December to February), further emaciated due to stressed from physical work (ploughing) and loss their body condition. Oxen in mixed crop livestock system serve for draft purpose from March to July of the year. The current finding is in line with Pannier *et al*. (2009) who report that energy source feed is used to activating Leptin protein production.

The sensory panelist evaluation in current finding is comparable with Adhikari (2013), on flavor for prime meat cuts (7.42) and choice cuts (7.24) and tenderness for prime cuts (8.66). However, the figure in these findings was higher compared with Gebeyehu *et al*. (2013), using trained sensory panelists rated (5.23) and (5.20) for beef tenderness and juiciness of Arsi cattle breed, respectively. Difference in sensory tenderness might be due to postmortem aging of Arsi cattle breed (Calkines and Sulivan, 2006).

The overall, mean value of WHC, initial pH, ultimate pH, and cooking loss were obtained 71.95, 6.55, 5.81, and 21.07 in the wet season and 73.82, 6.5, 5.62 and 9.99 in the dry season for beef of studied cattle, respectively. The result of WHC being low in wet than dry season. This might be due to lower WHC related lower body conformation in wet season than dry season. The overall mean cooking loss

of current finding in the wet season was comparable with the finding by Jama *et al*. (2008) who reported 23.9% cooking loss for Angus beef cattle in South Africa. However, higher values of 26.7% cooking loss was reported by Muchakilla *et al.* (2014) for Tanzania Shorthorn Zebu. These differences might be due to cooking procedures, feeding variations, and breed differences.

Effects of breeds and age

The effects of age, breed and season on beef eating quality are presented in Table 2. The analysis of variance indicated that breed, age and season interaction had showed significant effects (P<0.01) on instrumental tenderness, juiciness and flavor. The interaction between age and breed was significantly different (P<0.001) on juiciness, flavor, sensory tenderness and instrumental tenderness. The Harar cattle had better instrumental tenderness than *Bale* and *Arsi* breeds. This result might be due to the genetic and environmental difference. As age of cattle, increased value of WBSF also increased. This might be due to the presence of more collagen in older animal than younger once, which is less soluble during cooking. The current finding is in line with Guiusti *et al*. (2013) who reported that younger animals produced tender meat. The difference in instrumental tenderness across breeds might be due to differences in the type of feed and feeding strategies of the cattle's. Harar breed at Haramaya University was managed in feedlots for few days. However, the others breeds were finished on roughage after being kept on extensive grazing system. The current finding is in line with the finding of Muchakilla *et al.* (2014) who reported that beef from feedlot had better instrumental tenderness value compared to beef from grazing animals.

Age of animals has affected the sensory tenderness of beef (P<0.001). In general, the numerical mean value of Harar cattle had higher tenderness than other breeds. Kerry and Ledward (2008) reported that animals fed with concentrates had produced more tender steak than those fed with roughages. Juiciness was affected by the cattle breed types (P<0.01) but not with age of the animals and season (P >0.05). As the cattle gets aged, the juiciness of the meat has been decreased.

Table 2. Effects of breed, age, and season on beef sensory quality and WBSF in Ethiopian cattle

Factor	WBSF± SE	Sensory tenderness± SE	Juiciness ± SE	Flavor ± SE
Overall	34.51±1.50	7.21±0.06	7.19±0.05	7.25±0.06
Breed	***	Ns	**	**
Harar	24.72^{b}±1.42	7.10±0.11	7.40^{a}±0.08	6.97^{b}±0.11
Arsi	41.32^{a}±2.68	7.23±0.09	7.02^{b}±0.09	7.37^{a}±0.09
Bale	40.61^{a}±2.92	7.32±0.11	7.09^{b}±0.09	7.51^{a}±0.11
Age (years)	***	***	NS	***
< 5	23.15^{a}±1.72	7.74^{a}±0.08	7.40±0.08	6.69^{c}±0.11
5 - 7	28.70^{b}±2.60	7.17^{b}±0.08	7.21±0.10	7.23^{b}±0.11
7 - 9	38.25^{c}±2.49	7.13^{b}±0.12	7.13±0.08	7.44^{ab}±0.09
> 9	50.22^{d}±2.59	6.74^{c}±0.11	7.00±0.14	7.69^{a}±0.11
Age*Breed	***	***	***	***
Season	***	Ns	Ns	Ns
Dry	23.02^{b}±1.63	7.12±0.08	7.23±0.08	7.13±0.11
Wet	42.38^{c}±1.72	7.29±0.09	7.16±0.07	7.35±0.06
Age*Breed*S	***	NS	***	**

(p<0.05), ** (P<0.01), * (P<0.001), A= age, B= breed, S= season, SE = Standard error, NS=nonsignificant*

The analysis of variance indicated that age and breed have shown significant differences (P< 0.01) on flavor. The current finding is in line with Tran and Thu (2006) who reported that beef from older animal could produce a greater flavor due to having a high concentration of linoleic acid. The result

indicated that the flavor from Harar breed had the lowest score. This might be due to the stay of the Harar breed in a feedlot for few days before slaughter at Haramaya University compared to their breed counterparts. Muchakilla *et al*. (2014) reported that natural pasture grazed animal had good aroma score than animal finished in feedlot.

Effects of breeds and ages on water holding capacity, pH and cooking loss

Effects of ages, breeds and seasons on WHC, pH, and CL of beef are presented in Table 3. The breeds, ages and seasons interaction were shown strong significant difference (P<0.001) on WHC. The breeds type were highly significant variation (P <0.01) on WHC. This may be due to different breeds had different muscle structures. Seasons had shown strong significant difference (P < 0.001) on WCH. This might be happened due to cattle had different body condition at different seasons. The age had shown significant difference (P<0.05) on WHC. As the age of the cattle increased, WHC also increased. This might be due to as animal gets older the sarcolemma was contracted and extracellular muscle was decreased (Calkins and Sullivan, 2006). This result was in line with Warner *et al*. (2014) who reported that the WHC of meat is influenced by genetic, age and pre-slaughter animal stress. The season had shown strong significant difference (P< 0.001) on WHC. This happened due to cattle had different conformation at different season which commonly related to feed availability and exposed to physical work. This result was supported by Jorge and Rodrigo (2013) who reported that different feed types have a major influence on meat quality.

Table 3. Effects of breeds, age and seasons on WHC, pH, and cooking loss of beef

Factor	WHC± SE	Initial pH± SE	Final (24 hrs) pH± SE	Cooking loss ± SE
Overall	72.71±0.16	6.53±0.02	5.73±0.02	16.56±0.71
Breed	**	NS	NS	***
Harar	73.26^{a}±0.17	6.50±0.04	5.70±0.03	12.31^{b}±0.87
Arsi	71.81^{b}±0.35	6.57±0.03	5.76±0.04	18.60^{a}±1.31
Bale	72.94^{a}±0.29	6.51±0.04	5.76±0.05	20.21^{a}±1.20
Age	*	NS	NS	***
<5	72.14^{b}±0.38	6.51±0.05	5.65±0.04	16.47^{a}±1.00
5-7	72.94^{a}±0.24	6.50±0.04	5.74±0.04	16.93^{ab}±1.42
7-9	72.55^{ab}±0.37	6.58±0.04	5.76±0.06	19.06^{b}±1.73
>9	73.24^{a}±0.27	6.53±0.05	5.78±0.05	13.30^{c}±1.23
A*B	*	*	NS	***
Season	***	NS	***	***
Dry	73.82±0.21^{a}	6.50±0.04	5.62b±0.03	9.99^{b} ±0.75
Wet	71.95±0.19^{b}	6.55±0.03	5.81^{a}±0.03	21.07^{a}±0.68
A*B*S	***	*	*	***

(P<0.05), ** (P<0.01), * (P<0.0001), A= age, B= breed, S= season, SE= Standard error, NS=non significant*

The result of analysis variance indicated that ages, breeds and seasons interaction were not shown significant difference (P>0.05) on initial pH. However, numerically showed different number on seasons, breeds and ages groups. Generally, the mean value of initial pH was increased as age of animal increased. This might be due to the better body condition of the young cattle at slaughter than aged once. This lower body condition implicated that lower energy reserve, which exposed them for faster exhaustion of glycogen consequently resulting in relatively lower initial pH (Adzitey, 2011). The season had shown strong significance difference on (P<0.001) on ultimate pH. This might be happened due to environmental difference made chronic stress on beef cattle during beginning of wet season due to passing through in long dry periods, water scarce and physical work (Adzitey and Nurul, 2011).

The ages, breeds and seasons interaction were indicated strong significant difference (P<0.01) on CL. The decrease in cooking loss as aging increased was expected since enzymatic reactions by endogenous enzymes, such as collagenase, which are produced by bacterial within beef or by ionic solubilisation, progresses at faster rates aging increases. This might be also due to muscle (sarcomere) from a young animal was easily fragmented during cooking (Calkins and Sullivan, 2006). The current finding coincides with Jama *et al.* (2008**)** who reported that endogenous enzymatic reactions, such as collagenase disintegrated the myofibrillar proteins and made connective tissue thereby improving cooking loss.

Correlation of sensory evaluation with instrumental tenderness

Pearson correlation of sensory evaluation with instrumental tenderness, WHC, pH and cooking loss are presented in Table 5. There was a negative strong correlation (r= -0.48) on an instrumental tenderness with sensory tenderness and negative correlation (r=-0.27) on juiciness with instrumental tenderness. Similarly, the negative correlation was reported by Caine *et al*. (2003) on juiciness and instrumental tenderness (r= - 0.61). The strong positive correlation (r= 0.6) was detected on flavor and instrumental tenderness (WBSF), however negative correlation between flavor and sensory tenderness (r=.-0.66). This value was happened due to behaviors of data between flavor and sensory tenderness. Puente *et al*. (2015) reported similar result on cooking loss was correlated to WBSF (r = 0.44).

Analysis of result revealed that there were moderate relation between instrumental and sensory evaluation. The moderate/weak relationship between sensory evaluation and instrumental measurements of meat tenderness is generally accept. The first is the lack of precision arising from the use of sensory panelists because of the subjective nature of the measurements, e.g., the ability of panelist differing scales of perception for tenderness due to lack of exposure for meat in their daily food menu. In Ethiopian, there were no specialist meat sensory panelists as coffee quality panelist. Therefore, the moderate/weak correlation relation between sensory panelist and instrumental tenderness were expected. This finding is in line with Savell *et al.* (2011) who reported that a weak relationship between instrumental measurements and sensory evaluations. Similarly, Van Wezemael *et al*. (2013) reported the reason for weak correlations between sensory evaluations and instrumental tenderness as panelists experience, personal preferences, eating habits and social influences

Table 5. Pearson Correlation between sensory eating qualities with instrumental tenderness, WHC, pH, and cooking loss

Variable	WBSF	ST	JC	FVL	pHi	WHC	pHu	CL
WBSF	1							
ST	-0.48*	1						
JC	-0.27	0.10	1					
FVL	0.60**	-0.66**	-0.22	1				
pHi	0.14	-0.24	0.04	0.24	1			
WHC	-0.33	0.23	0.23	-0.21	-0.26	1		
pHu	0.56**	-0.57**	-0.05	0.58**	0.37	-0.40*	1	
CL	0.42*	-0.31	-0.68*	0.38	0.23	-0.58**	0.62**	1

**** (P<0.0001), ** (P<0.001), * (P<0.05), NS= non-significant, WBSF= Warner*

Conclusions and Recommendations

It is concluding that age, breed, and season were highly significant affect on eating quality parameters. The beef from Harar breeds had exhibited good eating quality based on tenderness and juiciness than other breeds but lower flavor. This might be due to finishing feed difference. As the age of animal increases the sensory tenderness and juiciness is decrease but instrumental tenderness and flavor increase. In study location good beef quality beef was produced at dry season (last September to December) due to good conformation of cattle during the onset dry season because of availability both quantity and quality feed resource, rest of draft cattle for a long time without physical work, less cold and warm weather stressInstrumental tenderness and sensory tenderness had negative relation (r= -0.48). Juiciness had weak negative relationship with WBSF (r= -0.27). Flavor had strong positive relationship with WBSF (r= 0.6). Generally, from this study it is realized that quality evaluation is essential for the development of the beef sector in Ethiopia. It is also observed that beef quality of young animals is high in quality and hence promotion of beef production at the right age should be promoted in Ethiopia. There is clear difference of breed in meat quality, indicating farmers need to fatten best breeds for beef production based on selection.

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Current Status of Camel Dairy Processing and Technologies: A Review

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Abstract

Camel milk is unique because it is not easily processed into different dairy products that are common for bovine milk. It lacks β-lactoglobulin and has low κ-casein that hinders processing of camel milk into different dairy products. Hence it needs different processing technologies to produce camel dairy products. Attempts have been made to produce dairy products from camel milk such as feta cheese, soft brined cheese, and fermented milk that have been produced at laboratory level. Camel milk powder has also been produced and marketed in Middle East and beyond. Currently there are tremendous progresses in camel dairy technologies that pave the way for production of different camel dairy products at small, medium and large scale in the near future in East and North Africa, and Middle East countries. Haramaya University has made tremendous progress in camel dairy technology in collaboration with Danish Universities during the last five years. During this period a number of experiments were conducted in wide areas from preservation of camel milk using lactoperoxidase system activation by addition of hydrogen peroxide (H_2O_2) and thiocyanate as well as by using H_2O_2 producing lactic acid bacteria (LAB) to metagenomic characterization of LAB isolated from spontaneously fermented camel milk. Therefore, this review paper is going to present outcome of researches conducted by Haramaya University in collaboration with University of Copenhagen and Technical University of Denmark.

***Key words**:* camel milk, dairy products, dairy technology

Introduction

Camel (*Camelus dromedaries*) the most climate resilient livestock in East Africa in general and in Ethiopia in particular, and plays significant role in the livelihood of pastoral and agro-pastoral communities in the region. Camel produces milk consistently despite frequent and severe drought and climate change. Camel milk is produced throughout the year and there is plenty of camel milk production during wet season, and this milk is mainly used for home consumption and the rest milk spoiled due to lack of access to market especially where there is no infrastructure for milk transportation and marketing.

With regard to processing of camel milk, it is similar to bovine milk in terms of gross composition but differs in terms of its detailed protein composition and colloidal structure. It lacks β-lactoglobulin, has small proportion of κ-casein and high proportion of β-casein in the casein micelles. The colloidal structure is also different with larger casein micelles and smaller fat globules.The different colloidal structure of camel milk, compared to bovine milk, means that most processing technology used for bovine milk cannot be directly applied for camel milk processing.

Even though camel milk is difficult to process it into different dairy products, its proteins have implications for human nutrition and are of interest for preparation of infant food formula and functional foods. It is also proposed for health benefits including inhibition of the angiotensin converting enzyme, antimicrobial and antioxidant properties as well as an antidiabetogenic effects. Therefore, the objective this review paper is to assess current technological advances in the area of camel dairy technology and future prospective to produce camel dairy products.

Preservation of Raw Camel Milk

Milk needs to preserved from microbial multiplication and spoilage by cooling the milk within two hours after milking and using other preservation methods such as lactoperoxidase system where there is no electric power supply. Lactoperoxidase system (LPS) was tested for camel milk preservation by Bekele Amenu *et al.* (2017) using hydrogen peroxide and thiocyanate, and found that chemical activation of LPS extended the shelf life of camel milk for up to 12 hrs at 30ºC. Moreover, LPS activation exhibited a bacteriostatic effect against coliform count (CC) and total bacterial count (TBC) and significantly ($P<0.05$) decreased the rate of growth of *S. aureus* and *E. coli* counts.

To avoid the use of chemical preservation of milk using LPS, six H_2O_2 producing strains of lactic acid bacteria (LAB) isolated from camel milk collected from Eastern Ethiopia were evaluated for H_2O_2 excretion (Dakalo Dashe, 2018). Theses LABs were *Lactococcus lactis* 22333, *Weissella. confusa* 22308, *W. confusa* 22282, *W. confusa* 22296, *S. infatarius* 22279, *S. lutetiensis* 22319. The quantification of H_2O_2 by titration method showed *W. confusa* 22282 to produce significantly the highest amount (302.10±20.55 mg/L) of H_2O_2 in MRS broth. *W. confusa* 22282 was selected as best strain to activate the natural antimicrobial system in camel milk. The activation of LPs by *W. confusa* 22282 significantly reduced ($P<0.05$) the rate of lactic acid development and microbial growth (TBC and CC) in raw camel milk at ambient temperature (18-24ºC) and *E. coli* up to 18 hours of storage. Therefore, production of H_2O_2 by *W. confusa* 22282 can activate the LPs and extend the storage stability of raw camel milk up to 18 hours at ambient temperature (Dakalo Dashe, 2018).

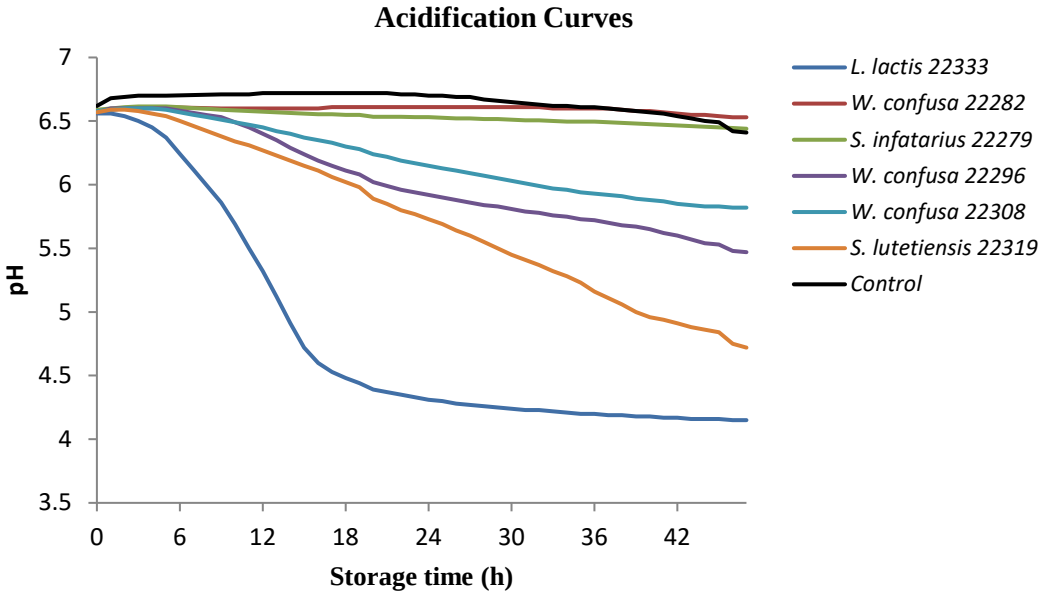

Figure 1. Acidification curves for H_2O_2 producing LAB strains in pasteurized camel milk (21-23 ºC)

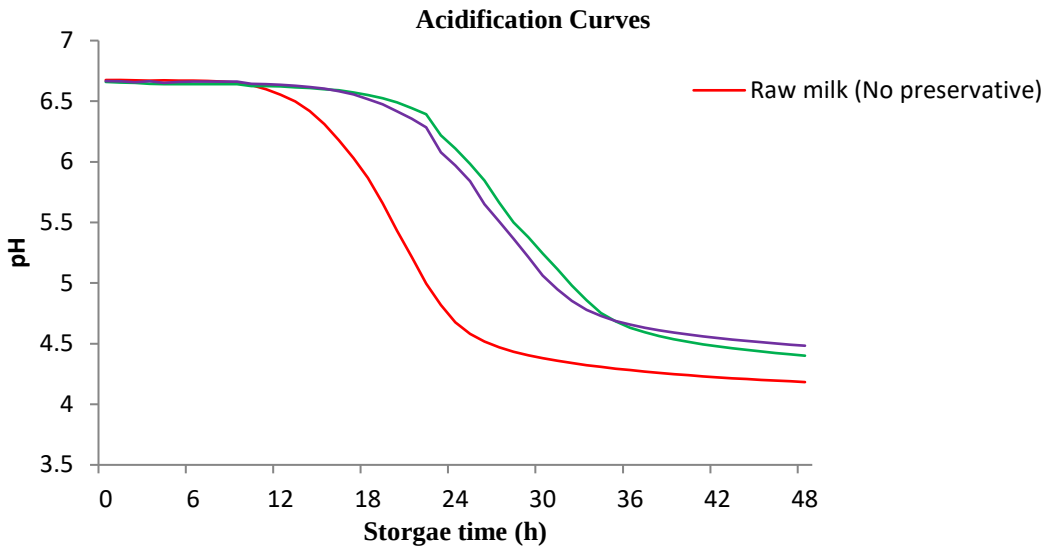

Figure 2. Acidification curves for *W. confusa* 22282 and H_2O_2 activated LPS and non-activated raw camel milk at room temperature.

Provision of appropriate cooling facilities and utilization of renewable energy technologies such as solar energy for milk processing are identified as possible intervention strategies to enhance milk marketing. Solar cooling system can be used to cool camel milk in rural settings where grid electric supply is unavailable. Hence, use of solar energy cooling system and cold chain could solve the limitation of camel milk marketing and spoilage. It can be used by organizing camel milk producers into camel dairy groups and/or cooperatives to establish market chain thereby to promote camel milk marketing. Solar milk cooling is effectively used in Kenya. This clean energy solution can increase dairy farm productivity and income by significantly decreasing milk spoilage. Effective cold-chain storage lowers bacteria count and improves milk quality for consumers. These improvements played a major role in the livelihoods of approximately one million smallholder dairy farming families in Kenya.

In Ethiopia access to electric power has reached 57%, while the number of households that are connected through the national electric power grid system has reached to 2.8 million (African News, 2018; http://www.xinhuanet.com/english/2018-04/20/c_137125761.htm). But only 26.5% of the rural population have access to electricity and the majority of the population particularly in pastoral and agro-pastoral areas do not have access to electricity. According to the World Bank, Ethiopia has the second highest available energy generation capacity in the Sub-Saharan African region, with nearly 100 percent coming from renewable energy generation (mostly hydropower), and vast and mainly untapped solar, wind, and geothermal clean energy resources. Solar energy is considered as the best option to reach the rural community in remote settings, particularly where there are high camel milk production and potential.

Moreover, mobile milk processing plant could be adapted for camel milk producing pastoral and agro-pastoral areas of the country. Mobile milk processing plants could be used where there is huge camel milk production but lack access to market due to distance from target market and lack of appropriate infrastructure. The mobile plant could produce different dairy products that are shelf stable and enable the producers to supply milk products to a wider area market. The mobile plant can be flexible to move from place to place depending on the mobility of camel herds. This plant can be integrated with solar energy milk cooling and processing facilities.

Effect of Heat Treatment on Whey Proteins Composition

Almaz Genene *et al.* (2019) evaluated the effect of heat treatment on whey protein composition and found that the amount of NPN in camel milk was not significantly (P>0.05) affected by heat treatment, however this was the case for bovine milk where the amount of NPN increased as the heat treatment intensity increased. This could be as a result of the presence of heat-induced protein degraded products (Kappeler, 1998; El-Agamy *et al*., 2009; Felfoul *et al*., 2016).

The amount of whey protein nitrogen in camel milk decreased significantly (P<0.05) at more severe heat treatments (90℃/5 min) and subsequently, the total whey protein denaturation increased. Similar results were also observed for bovine milk (Table1). Rynne *et al.* (2004) also indicated the level of total whey protein denaturation increased from 2.8%, to 34% due to increasing of pasteurization temperature from 72 ℃ to 87 ℃/26 sec. The denaturation percentage of whey protein in camel milk appears to be lower than for bovine milk which could be due to the relatively higher heat resistance of camel whey protein compared to bovine whey protein (El-Agamy, 2000; Felfoul *et al*., 2016).

Table 1. Effect of heat treatment on Total whey proteins (mg /100g) of camel milk and cow milk

Milk protein	Temperature/treatments (Mean +SD)						P value
	Raw	65^0C/30min	72^0C/30sec	75^0C/5min	85^0C/5min	90^0C/5min	
Camel							
NCN	110.75±1.76^{a}	108±1.41^{a}	105.2±1.13^{a}	98.8±1.97^{b}	87.25±3.88^{c}	74.00±2.82^{d}	***
NPN	29.75±0.70^{c}	30.6±0.77^{bc}	31.6±2.26^{abc}	32.6±1.34^{abc}	33.25±0.35^{ab}	33.90±0.14^{a}	ns
WPN	81.00±1.41^{a}	77.5±2.12^{ab}	73.70±0.98^{b}	66.50±3.53^{c}	54.00±4.24^{d}	40.10±2.68^{e}	***
WPD%	0	4.30±0.98^{ed}	9.00±2.82^{d}	18.0±0.2.82^{c}	33.37±4.06^{b}	50.50±2.51^{a}	***
Cow							
NCN	131.00±1.41^{a}	126±1.41^{ab}	122.50±0.7^{b}	115.0±4.24^{c}	87.55±3.60^{d}	76.00±2.82^{e}	***
NPN	25.50±0.70^{c}	26.00±1.41^{c}	27.7±0.49^{bc}	29.50±0.7^{abc}	31.65±1.9^{ab}	33.40±3.67^{a}	*
WPN	105.50±2.12^{a}	100.5±3.5^{ab}	95.00±1.41^{b}	85.50±7.77^{c}	55.90±4.87^{d}	42.60±0.84^{e}	***
WPD%	0	5.0±1.41d^{e}	10.07±2.92^{d}	22.00±5.65^{c}	47.00±0.56^{b}	60.75±0.07^{a}	***

Mean value with same superscripts letter in the same row were not significantly different at (P<0.05); NCN =Non casein nitrogen, NPN= Non protein nitrogen, WPN = whey protein nitrogen, WPD%= whey protein denaturation%, and ns = non-significant.

Effects of Heat Treatment on Individual Whey Proteins

Almaz Genene *et al*. (2019) also conducted SDS-PAGE for identification of individual protein (Figure 3) based on molecular weight. The presence of β-Lg was not found for camel milk (lane 8-13 in Figure 3) and similarly Kappeler *et al*. (2003); Hinz *et al*. (2012); Saliha *et al*. (2013); El haj and Freigoun (2015); Felfoul *et al*. (2016); Omar *et al*. (2016) reported that camel milk is deficient in β-Lg. However, in bovine milk β-Lg is the dominant whey protein (Farah, 1993; Hinz *et al*., 2012; Omar *et al*., 2016) and denatures at temperatures above 75 ℃.

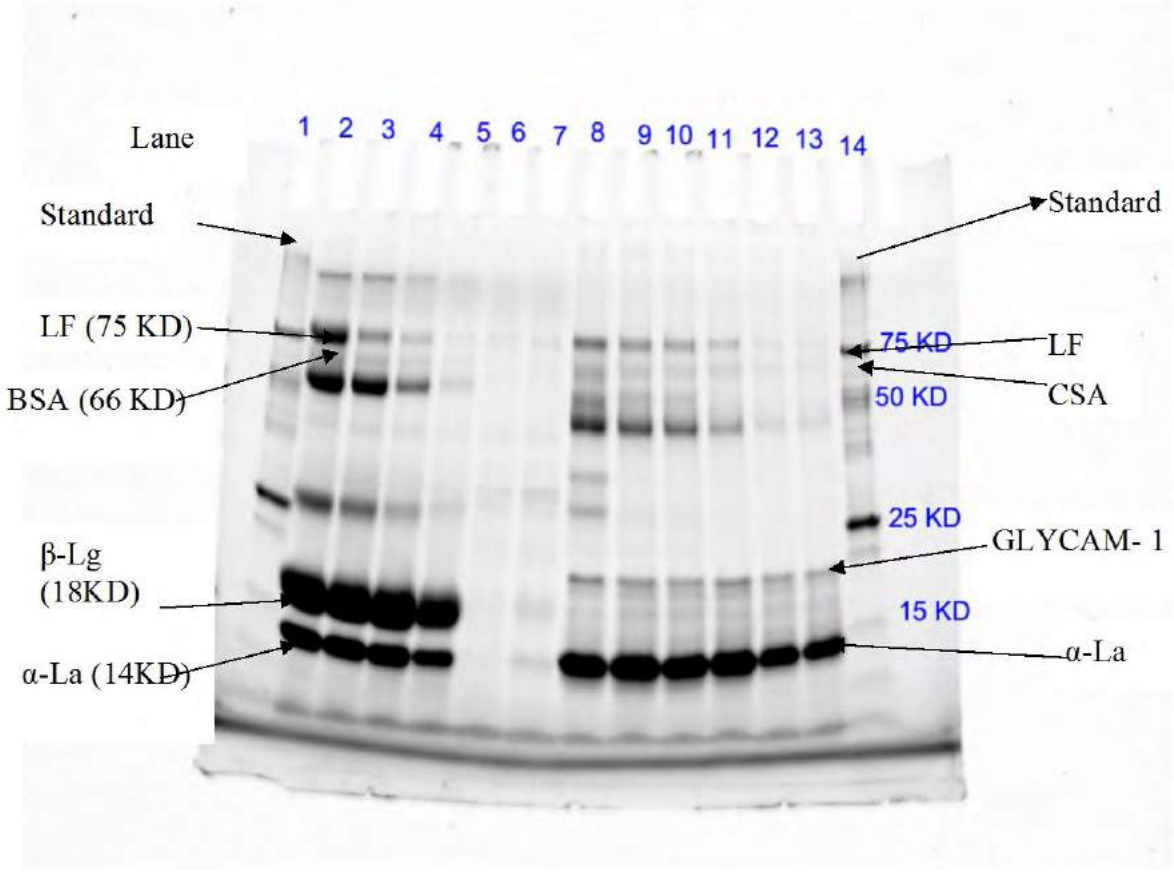

Figure 3: SDS-PAGE of whey proteins heated at different temperature for camel and cow milk. Lane 2 to 7 for cow milk and Lane 8 to 13 for camel milk stands for sample of Raw (reference sample), heated at 65 ℃/30 min, 72 ℃ /30sec, 75 ℃ /5min, 85 ℃ /5min and 90 ℃ /5min, respectively. Lane 1 and 14 are protein standards (Source: Almaz Genene et al., 2019).

Camel milk α-La band exhibits less visible change as heat treatment level increases (Figure 3). In contrast, bovine milk α-La band intensity was markedly reduced as heat treatment level increased and even totally disappeared at treatments of 85 ℃ /5min and 90 ℃ /5min (lane 6 and 7 in Figure 3). This might be due to the lack of β-Lg in camel milk, as β-Lg in bovine milk is known to interact with α-La and promote the interaction of α-La with casein micelles since α-La can form disulphide bridges but lacks the additional free thiol group of β-Lg, which initiates and enhances the interaction (Singh and Waungana, 2001; Donato and Guyomarch, 2009).

The association mechanism of denatured whey protein with casein micelles in camel milk has yet to be resolved in detail. However, according to Felfoul *et al.*, (2015) denatured α-La and camel serum albumin (CSA) is able to adhere on hot surfaces of dairy processing equipment either alone or interacting with casein micelles mainly κ-casein as β-Lg is responsible for cow milk. CSA might interact preferably with κ-casein (κ-CN) in camel milk during heat treatment, compared to α-la, due to the limited denaturation of α-la as temperature increases. For raw milk preconditioned to 40 ℃, a short gelation time and high gel strength was found and a heat treatment temperature of 65 ℃/30min or of 72 ℃/30s could be applied without compromising the rennetability of camel milk. A more intensive heat treatment, however, resulted in very low gel strength or no coagulation within 60 min. In order to understand the complex interaction between camel milk proteins during heat treatment, further research is required.

Manufacturing of Cheese from Camel Milk

Cheese is a popular dairy product produced and consumed by wide range of population in the world. Cheese is found to be a good method of preservation of perishable milk into a more shelf stable dairy products. Nutritionally, it is well commendable for supplying the required nutrients to our body. The taste is also acceptable and endured in the community. But manufacturing of cheese from camel milk have been hindered due to unique nature of camel milk in which camel milk lacks β-lactoglobulin and has low κ-casein and high proportion of β-casein that makes camel milk different from bovine milk. To overcome this obstacle a number of attempts were made where some of the attempts were successful and others failed to produce acceptable results. Yonas Hailu *et al.* (2017)

An experiment was conducted by Tekuam Walle *et al*. (2017) to investigate the effect of different levels of camel chymosin concentrations (40, 70, and 100 IMCU/L) on camel milk gelation properties and the influence of cooking/scalding of curd (uncooked and cooked at 55 ℃) on the characteristics of soft un-ripened cheese made from camel milk. The shortest gelation time was observed for camel chymosin concentration of 100 IMCU/L and 70 IMCU/L whereas the highest maximum gel firmness was observed for camel chymosin level of 40 IMCU/L. Similar authors concluded that, use of 70 IMCU/L chymosin concentration and cooking of camel milk curd could be suitable approaches for making of soft unripened cheese from camel milk Yonas Hailu *et al.* (2016) investigated the effect of temperature, pH, concentration of camel chymosin and addition of $CaCl_2$ on the hydrolysis of κ-CN and the coagulation kinetics of camel milk. They found that the rate of κ-CN hydrolysis was higher at 40 ℃ than at 30 ℃ and with increasing addition of chymosin (55 to 85 IMCU/L) and decreasing pH (6.6 to 6.0) and gelation of camel milk was initiated at >95% κ-CN hydrolysis. Likewise, hydrolysis of camel milk κ-CN can be maximized with increasing gelation temperature and camel chymosin concentration and decreasing pH. Increasing the gelation temperature causes camel milk casein gelation to start earlier, similar to the reported effect of reducing pH in bovine milk. Moreover, curd development from camel milk was improved by adding $CaCl_2$ at a rate of 0.02% w/ w.

Cheese Making and Compositional Changes

Production of cheese is a means of preserving valuable milk constituent (Adda *et al*., 1982) through a dehydration of coagulated milk using milk clotting enzymes (Fox, 1989; Lucey, 2008) and this process is reported to be difficult and tedious for camel milk (Bornaz *et al*., 2009; Ramet, 2001). However, the advent of coagulant camel chymosin with high specificity to κ-CN cleavage has provided success stories. Recently Yonas Hailu *et al*. (2018) conducted an experiment to evaluate manufacturing of soft brined cheese using camel chymosin and found the composition of soft brined cheese made from camel milk showed significant ($P < 0.05$) differences in the first 20 days of ripening. Salt in moisture (S/M) of camel milk cheese significantly ($P< 0.05$) increased with increase of NaCl % (w/w) and salting time (Table 2). Cheese making with 85 IMCU/L coagulant resulted in a higher degradation of caseins than was obtained with 55 IMCU/L coagulant. Similar to the case for brined cheese from bovine milk, β-CN in in brined camel cheese was degraded in later stage of ripening while αS1-CN remained more intact.

Table 2. Change in physicochemical properties and salt in moisture of soft brined cheese made from camel milk during ripening.

Day	Cheese pH	Total solids (%)	Ash (%)	Fat (%)	Protein (%)	S/M of 2% brine	S/M of 5% brine
0	4.91±0.05^{a}	45.02±1.09^{a}	1.52±0.20^{b}	24.50±0.56^{a}	19.29±0.92^{a}	0.42±0.15^{c}	0.46±0.15^{c}
20	4.49±0.05^{b}	39.83±1.09^{b}	3.09±0.20^{a}	21.94±0.56^{b}	17.81±0.84^{ab}	0.25±0.15^{b}	6.67±0.15^{a}
40	4.44±0.06^{b}	41.27±1.09^{ab}	3.22±0.20^{a}	22.19±0.56^{b}	15.53±0.84^{b}	0.74±0.15^{b}	6.68±0.15^{a}
60	4.50±0.05^{b}	39.92±1.09^{b}	3.13±0.20^{a}	22.75±0.56^{b}	14.68±0.98^{b}	4.19±0.15^{b}	6.45±0.15^{a}

Values are least square means ± standard error (SE) (n=3) physicochemical properties of four cheeses and salt in moisture (S/M) of two cheeses. Means with the same superscript letter within a column are not significantly different (P > 0.05). (Source: Yonas Hailu et al. (2018).

Proteolysis in Soft Brined Cheese

One of the major phenomenon that occur during cheese ripening is degradation of casein (Fox, 1989; Sousa et al., 2001) as a result of residual coagulant, enzyme from starter culture, nonstarter culture and milk enzymes like plasmin (Fox, 1989; Sousa *et al*., 2001). The degraded soluble components of caseins diffuses to the brine during ripening (Guinee, 2004; Guinee and Fox, 2004; Anifantakis, 1996). The amount of total nitrogen in brined camel milk cheese significantly (P < 0.05) decreased during the ripening period**.** Similarly Hayaloglu *et al*. (2002); Michaelidou *et al*., (2005); Anifantakis (1996) reported that soluble nitrogen fraction decreased during ripening of soft brined cheese made from milk of other species due to migration of soluble components to brine and the level in the cheese will be reduced. Hence length of ripening time of cheese affects the biochemical changes in cheese (Pappas *et al*., 1996).

Table 3. Comparison of the time to reach pH 4.6 of commercial starter cultures inoculated into camel and bovine milk

Group	Culture	Camel milk Time to pH 4.6 (h:min)			Cow milk Time to pH 4.6 (h:min)		
		30 ℃	37 ℃	42 ℃	30 ℃	37 ℃	42 ℃
I: Mesophilic cultures	R-704	12:40^{c}	16:48^{b}		8:25de	9:35^{d}	
	R-704	8:10de	16:05^{b}		5:55^{f}	7:35^{ef}	
	CHN-22	12:35^{c}	21:15^{a}		9:10de	19:45^{a}	
II: Blend of mesophilic and thermophilic cultures	RST-743	7:55^{ef}	7:52^{ef}	7:23^{f}	7:40^{f}	5:05^{g}	4:50^{g}
	XPL-2	13:40^{b}	15:08^{a}	9:58^{d}	11:20^{c}	8:54de	7:30^{f}
III: Thermophilic cultures	Yoflex mild 1.0		8:30^{a}	8:27^{a}		4:30^{cde}	3:45^{ef}
	YF-L904		8:42^{a}	8:37^{a}		4:39^{ed}	4:03^{def}
	STI-12		5:32^{b}	5:10^{bc}		4:18^{def}	3:35^{f}

Means with the same letter across columns and rows within group are not significantly different (p > 0.05) (Source: Tesfamariam Berhe et al., 2018)

Birhanu Bekele *et al*. (2019) investigated the effect of starter cultures on the physicochemical properties, texture, and consumer preferences of soft white cheese (SWC) made from camel milk (Table

4). The experiment employed five starter cultures; one thermophilic (STI-12), two blended (RST-743 and XPL- 2), and two mesophilic (R-707 and CHN-22) cultures. Camel milk inoculated using STI-12 and RST-743 cultures resulted in faster acidification than XPL-2, R-707, and CHN-22 cultures. Camel milk SWC made using STI-12 and CHN-22 cultures gave lower pH (4.54) and titratable acidity (0.59), respectively, whereas R-707 culture resulted in high cheese yield (13.44 g/100 g). Considering curd firmness, cheese yield, compositional quality, and textures, STI-12, RST-743, and R-707 cultures were found to be better for the manufacture of camel milk SWC.

Table 4. Physicochemical properties of soft cheese made from camel and cow milk

Parameters	Values (Mean ± SD)					
	STI-12 ($37^{O}C$)	RST-743 ($37^{O}C$)	R-707 ($30^{O}C$)	XPL-2 ($30^{O}C$)	CHN-22 ($30^{O}C$)	P-values
Camel						
pH	4.54 ± 0.17^{c}	4.76 ± 0.12^{bc}	5.04 ± 0.12^{ab}	4.92 ± 0.05^{ab}	5.20 ± 0.06^{a}	*
Acidity (%)	1.09 ± 0.15^{a}	0.87 ± 0.02^{b}	0.71 ± 0.07^{bc}	0.73 ± 0.08^{b}	0.59 ± 0.02^{c}	**
Yield (kg/100kg)	9.43 ± 1.85^{b}	9.77 ± 0.98^{b}	13.44 ± 0.09^{a}	10.18 ± 0.27^{b}	9.50 ± 0.34^{b}	*
Fat (%)	18.71 ± 0.56^{b}	20.91 ± 0.82^{a}	18.89 ± 0.76^{b}	17.99 ± 0.45^{b}	18.74 ± 0.57^{b}	*
Protein (%)	16.29 ± 0.11^{a}	17.49 ± 1.73^{a}	12.18 ± 0.10^{b}	16.33 ± 0.55^{a}	11.12 ± 0.02^{b}	**
TS (%)	40.40 ± 0.46^{ab}	43.44 ± 2.80^{a}	35.77 ± 0.68^{cd}	38.54 ± 0.7^{bc}	34.76 ± 0.26^{d}	**
Ash (%)	2.21 ± 0.14^{ab}	2.40 ± 0.27^{a}	1.79 ± 0.23^{bc}	2.20 ± 0.31^{ab}	1.27 ± 0.04^{c}	*
Cow						
pH	4.62 ± 0.01^{bc}	4.81 ± 0.02^{ab}	4.52 ± 0.16^{c}	4.64 ± 0.03^{bc}	4.95 ± 0.04^{a}	*
Acidity (%)	0.95 ± 0.01^{ab}	0.81 ± 0.10^{bc}	1.04 ± 0.07^{a}	0.96 ± 0.01^{ab}	0.74 ± 0.02^{c}	*
Yield (kg/100kg)	18.08 ± 0.77^{a}	16.23 ± 0.81^{bc}	15.60 ± 0.39^{c}	17.59 ± 0.12^{ab}	15.00 ± 0.04^{c}	**
Fat (%)	18.48 ± 0.45^{a}	17.65 ± 0.15^{ab}	18.02 ± 0.77^{a}	16.4 ± 0.36^{bc}	15.84 ± 0.65^{c}	*
Protein (%)	16.18 ± 0.03^{a}	13.17 ± 0.07^{c}	15.63 ± 0.14^{a}	13.4 ± 0.28^{bc}	13.83 ± 0.42^{b}	***
TS (%)	40.53 ± 1.45^{a}	35.72 ± 0.27^{b}	38.87 ± 0.49^{a}	34.88 ± 0.13^{b}	34.81 ± 0.74^{b}	**
Ash (%)	2.73 ± 0.17^{a}	1.80 ± 0.03^{c}	2.22 ± 0.14^{b}	2.39 ± 0.02^{b}	2.20 ± 0.10^{b}	**

Means with different superscript letters within the same row showed significant differences ($P<0.05$) among soft cheeses made using different commercial cultures. DM = Dry matter

Significant differences ($P<0.05$) in physicochemical properties was observed between SWC made from camel and cow milk in which pH of SWC made from camel milk using XPL-2 and CHN-22 cultures had significantly higher cheeses pH values than that of XPL-2 and CHN-22 cultures used for manufacturing of SWC from cow milk, respectively. This vitiation might be attributed to the characteristics of milk types that the acidification of camel milk was reported to take longer time than bovine milk (Al and Kanhal, 2010). camel milk soft cheeses made by cultures RST-743 and CHN-22 had significantly higher fat than that of cow milk soft cheeses made using cultures RST-743 and CHN-22.

4.4. Camel and Cow Milk Cheeses Whey Compositions

Whey can be used for food and pharmaceutical products. Whey contains some potentially valuable and useful components in the form of functional food proteins, lactose, vitamins and minerals. Trials have shown that camel milk whey may be used to make acidified drinks. These drinks have an excellent nutritive value because of the presence of essential amino acids, lactose, lactic acid, vitamins and minerals.

Birhanu Bekele *et al.* (2019) found significant differences ($P<0.05$) among cheeses whey composition in SWC made from camel milk. Fat composition of 1.12±0.09 in camel milk soft cheese whey made using culture CHN-22 had significantly higher (P<0.05) than that of camel milk soft cheeses made using cultures STI-12, RST-743, R-707 and XPL-2. Similarly, Konuspayeva *et al*. (2016) found 1.10 ± 0.26 fat in brine-salted camel milk soft cheese whey and Shahein *et al*. (2014) found 1.5% fat for camel milk cheese whey.

Camel milk soft cheeses whey composition made using cultures XPL-2 and CHN-22 had higher ($P<0.05$) protein than that of camel milk soft cheeses made using cultures STI-12, RST-743 and R-707. Lactose composition of camel milk cheeses whey made using culture XPL-2 was higher ($P<0.0001$) than in camel milk soft cheeses whey made using cultures STI-12, RST-743, R-707 and CHN-22. The lactose content of camel milk soft cheese whey made by culture XPL-2 is in agreement with the report of Konuspayeva *et al*. (2016) who stated 4.26 ± 0.18 lactose of brine-salted soft camel cheese whey. Camel milk soft cheese whey made using cultures XPL-2 and CHN-22 had higher ($P<0.0039$) total solids and ash content than that of cultures STI-12, RST-743 and R-707 used for camel milk cheese whey total solids and ash (Table 5). However camel milk soft cheeses whey made using cultures STI-12, RST-743 and R-707 had significantly the same camel milk cheese total solids; nevertheless, soft cheeses made by cultures RST-743 and XPL-2 had lower camel cheese ash than that of cultures STI-12, R-707, and CHN-22 used for camel milk soft cheeses whey ash composition. The value of total solids in XPL-2 agree with the finding of Konuspayeva *et al*. (2016), who reported 7.54 ± 0.11 total solids of brine-salted camel milk soft cheese.

Table 5. Camel and cow milk cheeses whey compositions

Parameters (g/100g)	Values (Mean ± SD)					P-values
	STI-12	RST-743	R-707	XPL-2	CHN-22	
Camel						
Fat	0.57±0.01^{b}	0.34±0.02^{b}	0.58±0.04^{b}	0.57±0.37^{b}	1.12±0.09^{a}	***
Protein	0.65±0.05^{b}	0.66±0.02^{b}	0.68±0.03^{b}	0.80±0.04^{a}	0.86±0.04^{a}	*
Lactose	3.88±0.09^{d}	4.39±0.02^{b}	4.10±0.04^{c}	4.95±0.02^{a}	4.08±0.09^{c}	***
TS	6.74±0.13^{b}	6.58±0.01^{b}	6.96±0.20^{b}	7.46±0.31^{a}	7.90±0.16^{a}	*
Ash	1.39±0.09^{c}	1.18±0.03^{d}	1.59±0.07^{b}	1.13±0.04^{d}	1.83±0.10^{a}	***
Cow						
Fat	0.22±0.01^{ab}	0.16±0.02^{bc}	0.13±0.02^{c}	0.26±0.03^{a}	0.15±0.04^{bc}	*
Protein	0.90±0.02^{a}	0.94±0.04^{a}	0.99±0.13^{a}	0.67±0.01^{b}	0.87±0.01^{a}	*
Lactose	4.84±0.08^{b}	5.01±0.15^{b}	4.89±0.03^{b}	5.28±0.01^{a}	5.25±0.03^{a}	*
TS	6.79±0.07^{bc}	6.90±0.03^{ab}	6.60±0.21^{c}	7.15±0.04^{a}	6.89±0.05^{ab}	*
Ash	0.82±0.01^{ab}	0.79±0.11^{b}	0.58±0.01^{c}	0.93±0.01^{a}	0.61±00^{c}	**

Means with different superscript letters within the same row showed significant differences (P<0.05) among camel and cow cheese whey compositions.

4.5. Rheological Texture of Soft Cheeses Made from Camel and Cow Milk

Figure 4 indicates the compression curves of force verses time of soft cheeses made from camel milk. In this experiment, it was observed that soft cheese made from camel milk using culture RST- 743 resulted in higher resistance to fracture values compared to cheese made from cultures STI-12, R-707, XPL-2 and CHN-22. Camel milk soft cheeses sample made with cultures RST-743 and STI-12 had lower moisture and higher protein contents that showed a higher resistance to fracture and less breakable compared to cheese made using cultures R-707, XPL-2 and CHN-22.

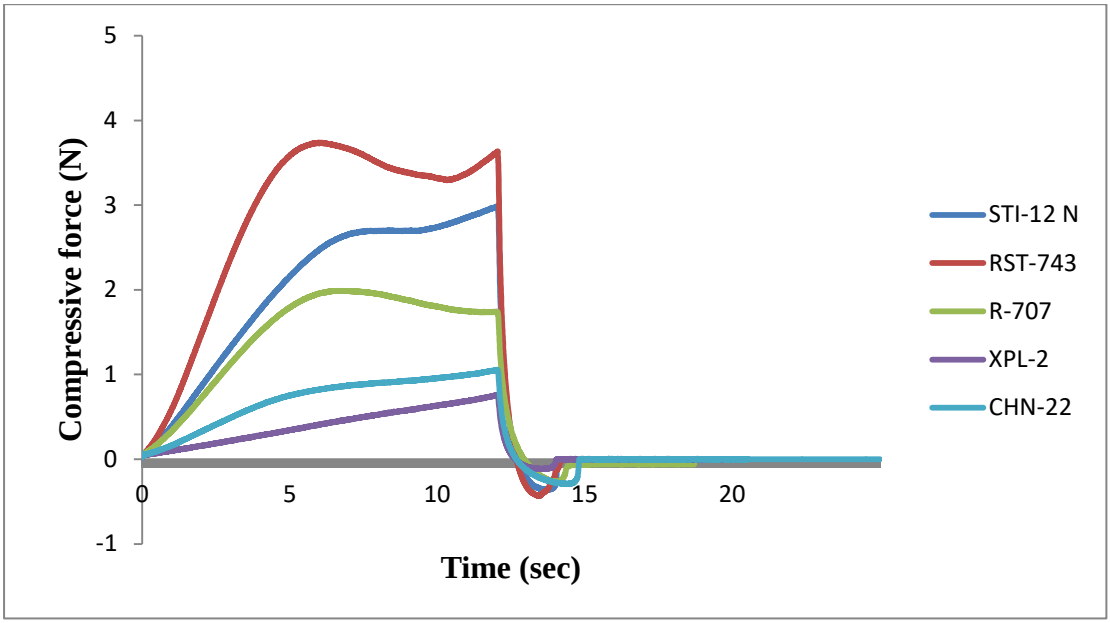

Figure 4: Compression curves of cheeses made from camel milk using five different cultures

Camel milk soft cheese sample made with culture XPL-2 had similar moisture value with culture STI-12 and protein value with cultures RST-743 and STI-12; however, due to the fact that XPL-2 undergo a syneresis during the instrumental test, thus XPL-2 may be showed less resistance to fracture. The variations of these textures could be attributed to the moisture and protein contents of the cheese samples. The moisture and protein contents of the cheese affected the rheological characteristics of the cheeses (Bongiolo *et al*., 2014).

Rheological textures of firmness, brittleness and stickiness for the final processed soft cheeses were measured using micro-stable texture analyzer. The instrumental analysis led to observe a difference in firmness among camel milk cheeses produced with different cultures. The result indicates that camel milk soft cheese made with culture RST-743 gave significantly higher firm (P<0. 0.01) and brittle (P<0.05) values than camel soft milk cheese made using cultures XPL-2 and CHN-22 (Figure 5 and 6, respectively).

These variations may be due to the fact that during manufacture the moisture and calcium contents of cheese can alter the effect of pH on cheese texture. Higher-moisture content cheeses, at the same pH and salt content are less firm than their lower-moisture content. This has been attributed to the extent of swelling of casein sub-micelles with the increase in casein-to-moisture ratio. Consequently, even small variations in moisture content can have significant effect on cheese texture of fresh cow milk cheese (Gunasekaran, 2003). In addition to these, Guinee *et al*. (2001) investigated the effect of fat content on cheese microstructure and texture. They explained that increases in fat content resulted in smoother and softer cheese, and increase in casein content results in firmer cheese. It was also found that higher fat and water content tends to weaken the protein structure, and vice versa (Fox *et al.*, 2017)

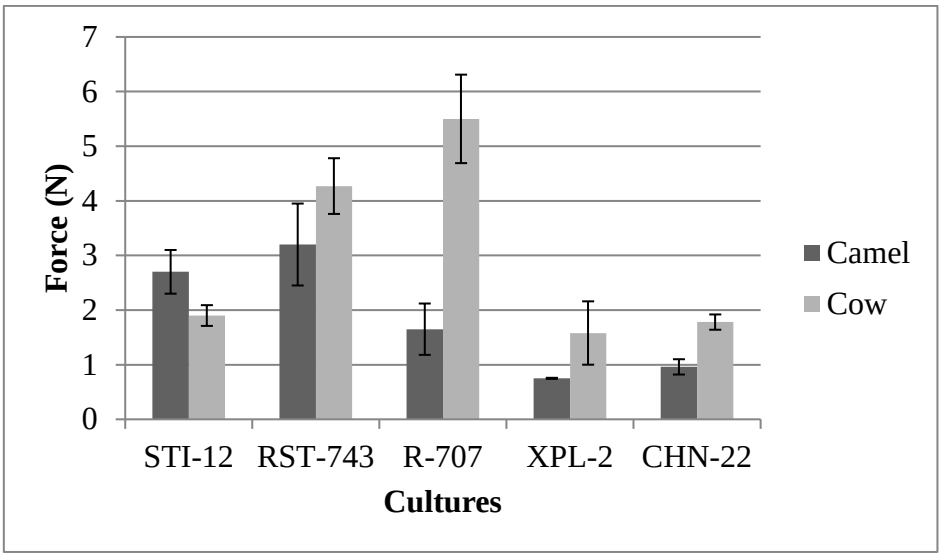

Figure 5. Camel and cow milk soft cheeses firmness

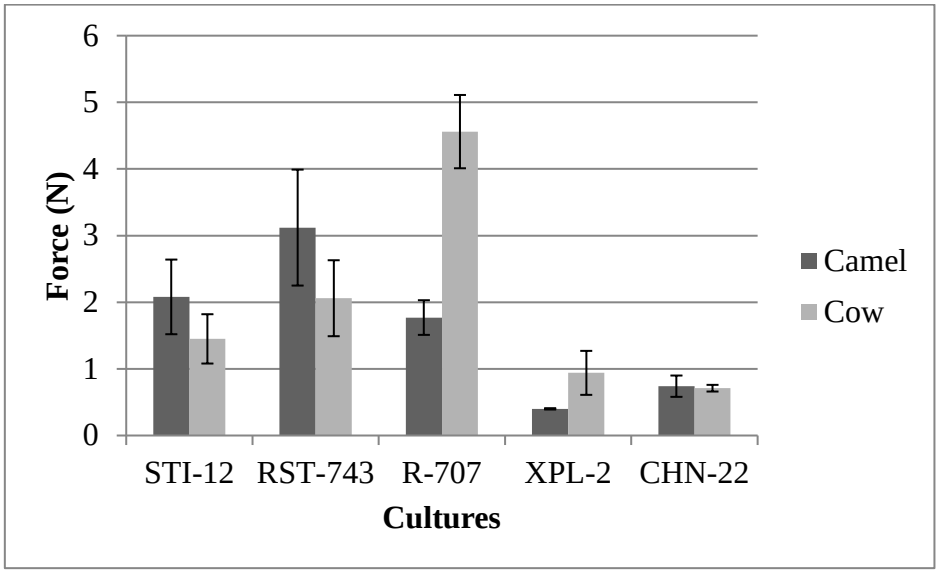

Figure 6. Camel and cow milk soft cheeses brittleness

4.6. Consumer Preference

Camel milk soft cheese made using culture XPL-2 had lower scores for appearance (P<0.001) and overall acceptance (P<0.0001) than that made using cultures STI-12, RST-743, R-707 and CHN-22 (Table 6). On the other hand, camel milk soft cheeses made using cultures XPL-2 and CHN-22 had higher (P<0.0001) scores for aroma and taste as compared to camel milk soft cheeses made using cultures STI-12, RST-743 and R-707. These variations in flavor could be attributed to the inherent properties of an aromatic forming cultures inoculated in the milk during cheese-making.

Camel milk soft cheese made using R-707 had lower (P<0.0001) scored firmness than made using cultures STI-12, RST-743, XPL-2 and CHN-22; however, no significant difference (P>0.05) was observed in camel soft cheese made by cultures STI-12 and RST-743. In addition to camel milk soft cheese made with culture R-707, camel milk cheeses made using STI-12 and RST-743 had higher (P<0.0001) scores of crumbliness than that made using cultures XPL-2 and CHN-22.

Table 6. Consumer preference represents test scores of camel and cow milk soft cheeses

Milk Source	Parameters	(Mean ± SD)					
		STI-12	RST-743	R-707	XPL-2	CHN-22	S.L
Camel	Color	6.66±0.49^{a}	6.75±0.45^{a}	6.50±0.52^{a}	6.66±0.65^{a}	6.66±0.49^{a}	ns
	Appearance	6.33±0.65^{a}	6.16±0.57^{a}	6.41±0.66^{a}	5.25±0.86^{b}	6.00±0.42^{a}	***
	Aroma	5.16±0.38^{b}	5.08±0.51^{b}	4.83±0.57^{b}	6.25±0.62^{a}	6.58±0.51^{a}	***
	Taste	4.83±0.83^{b}	4.91±0.51^{b}	4.66±0.51^{b}	6.16±0.57^{a}	6.41±0.51^{a}	***
	Firmness	6.08±0.66^{a}	6.50±0.52^{a}	4.66±0.88^{b}	3.16±0.83^{c}	4.91±0.28^{b}	***
	Crumbliness	6..25±0.62^{a}	6.41±0.51^{a}	5.83±0.88^{a}	4.83±1.02^{b}	4.66±0.77^{b}	***
	Stickiness	5.66±0.88^{ab}	5.41±1.08^{b}	6.33±0.49^{a}	5.58±0.99^{b}	5.41±0.79^{b}	ns
	Overall acceptance	6.08±0.51^{a}	6.00±0.42^{ab}	5.80±0.38^{ab}	4.16±0.57^{c}	5.58±0.79^{b}	***
Cow	Color	6.50±0.52^{a}	6.25±0.75^{a}	6.33±0.49^{a}	6.08±0.51^{a}	6.08±0.51^{a}	ns
	Appearance	6.16±0.71^{a}	6.16±0.71^{a}	6.16±0.71^{a}	6.06±0.51^{a}	6.33±0.49^{a}	ns
	Aroma	5.16±0.38^{b}	5.08±0.51^{b}	5.08±0.51^{b}	6.25±0.62^{a}	6.08±0.28^{a}	***
	Taste	5.33±0.65^{b}	5.41±0.51^{b}	5.16±0.38^{b}	6.41±0.51^{a}	6.25±0.45^{a}	***
	Firmness	5.66±0.49^{b}	5.75±0.62^{b}	6.66±0.49^{a}	4.75±0.86^{c}	4.83±0.57^{c}	***
	Crumbliness	5.83±0.38^{b}	5.75±0.62^{b}	6.50±0.52^{a}	64.50±0.79^{c}	4.75±0.75^{c}	***
	Stickiness	5.16±0.71^{bc}	5.66±0.77^{b}	4.66±0.49^{c}	6.41±0.66^{a}	5.41±0.79^{b}	***
	Overall acceptance	5.83±0.38^{b}	5.66±0.65^{b}	6.41±0.51^{a}	5.08±0.79^{c}	4.66±0.49^{c}	***

Means with different superscript letters within a row are significantly different (P < 0.05); S.L= Significance Level, ns= non-significant.

The sensory scores of color, appearance and aroma of soft cheeses camel milk made using cultures XPL-2, R-707, and STI-12, respectively are in line with the report of Yonas Hailu *et al*. (2014), who found 6.50± 0.68, 6.17± 0.83, and 5.20 ±1.27 for color, appearance and aroma, respectively; however, the value of taste scored in cheese culture of XPL-2 is higher than 5.70± 1.09 cheese made using camel chymosin as a coagulant. On the other hand, the higher overall acceptance of sensory scored for STI-12 is lower than earlier literature of Yonas Hailu *et al*. (2014) who scored 6.47± 0.68 for soft unripened cheese made using camel chymosin as a coagulant. The variations in consumer preference could be attributed to the natural property of inculcated commercial starter cultures during acidification and curd making especially, compounds such as CO_2, diacetyl, and acetaldehyde are formed and attributed to the development of distinct textural and flavor properties of the cheeses (Walstra *et al*., 2006).

Fermented Camel Milk

Acidified milk drinks appear promising as do production of camel milk cheese. A number of attempts were conducted to manufacture fermented milk from camel milk. It was found that fermented camel has poor viscosity and consistence. The microbial communities in spontaneously fermented camel milk from eastern Ethiopia were characterized through metagenomic 16S rRNA sequencing and lactic acid bacteria were isolated with the goal of selecting strains suitable as starter cultures. The fermented camel milk microbiota was dominated either by Lactobacillales or by Enterobacteriaceae, depending on incubation temperature and the provider of the milk. Strains of species with a potential use as starter cultures i.e., *Lactococcus lactis*, *Lactobacillus plantarum*, and *Pediococcus acidilactici*, were isolated.

Fast acidifiers of camel milk have been isolated from the species of *Lc. lactis*, *P. acidilactici*, and *Streptococcus infantarius*. Gram-negative and potentially pathogenic microorganisms were common in spontaneously fermented camel milk, indicating the need for improved hygiene in camel milk production (Angelina *et al*., 2017).

UHT and Sterilized Camel Milk

Pasteurized camel milk appears straightforward and is used industrially, but ultrahigh temperature (UHT) and sterilization treatment of camel milk cause protein instability. Hence, research is needed to solve this problem. Currently Camelicious-Emirates industry for camel milk is producing UHT camel milk and marketing worldwide. It has a shelf life of 12 months if it is stored in cool and dry places. In Ethiopia on plant in Fafum town plans to manufacture UHT camel milk (personal communication).

Omar *et al*. (2011) tried to produce UHT camel milk and to this end they undertaken a number of experiments. They used different chemicals such as sodium hydroxide (NaOH) and calcium chloride ($CaCl_2$), κ-casein from bovine, sodium dihydrogen phosphate anhydrous, disodium hydrogen orthophosphate and Ethylenediaminetetraacetic acid disodium (EDTA) salt to stabilize camel milk proteins. Findings in this research showed that κ-casein and calcium content are expected to be the main factors affecting the low heat stability of camel milk protein. Furthermore, camel milk appears to belong to type A milk and has 2 stage of coagulation in proteins. Results also exhibited that camel milk could be converted from type A to type B and eliminate its minimum heat stability by adding κ-casein or EDTA. Results also showed that pH increase was beneficial for camel milk heat stability; however, very small change in pH could result in large effect on protein heat stability. Camel milk was shown to be sterilised if the pH increased to 7.0-7.2 or certain additives added. Some additives such as phosphate could be used in commercial scale to improve heat stability of whole camel milk.

The κ-casein is known to be the main factor stabilising casein micelle in milk. Its content in camel milk is lower than that in cow milk (Al Haj and Al Kanhal, 2010; Kappeler *et al.*, 2003) which makes it less stable. However, different concentration of κ-casein was added to camel milk to examine its effect on protein stability at different pH after sterilisation process. The addition of κ-casein was reported (Horne and Muir, 1990; Tessier and Rose, 1964) to increase the stability of cow milk type A at the pH range (6.7-6.8) of the minimum heat stability. Similarly, the addition of κ-casein at a concentration of 1 mg/ mL has shown to increase the heat stability of pre-adjusted camel milk protein type A at the pH range of the minimum heat stability of pH 6.8-6.9. The stabilising effect of κ-casein on heating at high temperature for milk proteins was reported (Van Boekel *et al.*, 1989) to protrude the hairy layers of c-terminal of κ-casein providing steric repulsion for casein which leads to increase heat stability. However, no effect on camel milk protein heat stability was noticed below this pH range. Further increase of κ-casein concentration to 2 mg/mL in camel milk type A has shown to increase the heat stability and eliminate the minimum heat stability at pH range 6.7-6.9, as compared to normal camel milk type A containing no added κ-casein.

EDTA was added to camel milk as a chelating agent to disrupt casein micelles (Lucey and Horne, 2009) by reducing calcium ion contents, as well as colloidal calcium phosphate (Udabage *et al.*, 2000) which led to casein micelle dissociation (Gaucheron, 2005) and consequently increased heat stability of camel milk. Different concentration of EDTA (2.0, 4.0 and 6.0 mmol/L) was added to determine its effect on camel milk protein heat stability after sterilisation process. The addition of 2.0 mmol/L was found to have no effect on camel milk heat stability at the studied range of 6.5-7.4. Further increase in EDTA

concentration to 4.0 or 6.0 mmol/L was shown to extend the heat stability range towards the acidic side from 7.0-7.3 to 6.6-7.4 for both concentrations, compared to normal camel milk containing no added EDTA. But the maximum heat stability was at the pH range 6.7-7.2 and 6.7-7.3, respectively; where proteins sedimentation of samples above or below these ranges were reversible except the natural pH of camel milk of 6.5 where protein sedimentation was irreversible. The addition of EDTA to camel milk type A at a concentration of 4.0 or 6.0 mmol/L was noticed to convert it to type B and eliminate its minimum heat stability due to calcium binding to EDTA.

Disodium phosphate is usually used during UHT treatment to prevent destabilisation of goat milk (Raynal-Ljutovac *et al.*, 2007). The addition of sodium phosphate at a concentration of 1 mmol/L to whole camel milk type A has broadened the stability range from 7.0-7.3 to 6.8-7.3. Moreover, added phosphate has no effect on camel milk stability below or above this range (pH 6.8-7.3). However, the maximum heat stability of camel milk containing phosphate at a concentration of 1 mmol/L was found to be slightly shifted to the acidic side at the pH range 6.8-7.1. Moreover, increasing sodium phosphate concentration to 2 mmol/L has further broadened the maximum heat stability range into 6.8-7.2. The presence of added phosphate in camel milk helps to decrease the effect of high temperature on pH decrease, in addition to increase the heat stability of camel milk at pH points (6.8 and 6.9), which was considered as minimum in normal milk containing no added phosphate. It is believed that added phosphate could increase the heat stability by binding soluble Ca2+ hence induced calcium phosphate precipitation on the micelle or lower calcium activity (Raynal-Ljutovac *et al.*, 2007). In another study, the calcium ion activity of concentrated milk was reported to decrease from 0.60 mmol/L to 0.27 mmol/L at 120°C, pH 6.5 when phosphate was added, while decreased from 0.83 mmol/L to 0.71 mmol/L when phosphate was not added (Nieuwenhuijs *et al.*, 1988).

Conclusion

The current status of camel dairy technology showed that there are satisfactory progresses that enable to process camel milk into different dairy products at small as well as at large scale which in turn will have positive impact on the livelihood of camel rearing community in East and North Africa. Heat treatment of camel milk resulted in less denaturation of α-lactalbumin compared to cow milk and camel milk serum albumin appears to be the most heat labile whey protein in camel milk. Moreover, a pasteurization temperature not exceeding 72 °C/30sec should be adopted for cheese making from camel milk. It is found that the difference in speed of acidification in bovine and camel milk is due to difference in proteolysis rather than the presence of inhibitory substance in camel milk. R-707 was found to be the best mesophilic culture and STI-12 the best thermophilic culture for camel milk fermentation. Production of UHT camel milk is feasible and being manufactured by some dairy plants in middle but it needs development of precise procedure and protocol. Currently there are about five dairy plants that are dedicated to process camel milk into different dairy products in Ethiopia (two in Somali Regional State, two in Afar Regional and one in Dire Dawa City Administration) and need to be supported by the scientific community and funding organizations.

The Ways Forward

Camel milk is marketed in raw state and there is a serious problem of lack of cold chain. The safety and quality of camel milk is also of substandard and does not meet standards set by Quality Standards Authority of Ethiopian. To this effect most of the camel milk is not marketed in pastoral and agro-pastoral areas of the country. To overcome these problems introduction of cold chain in the form of solar milk cooling will play paramount importance. Hence concerned organizations or institutions

should step up and take immediate action of introduction of cold chain by identifying potential camel milk production areas. Moreover, quality and quantity camel milk production need to be promoted through introduction of improved and affordable milking equipment such as mazzi milk cans. The other strategies that could solve and reduce camel milk spoilage is use of mobile milk pasteurization facilities that can easily transported and planted in remote areas. This could be augmented by solar energy generation for power supply that could be used both for mobile pasteurization and milk cooling refrigerators. Alternatively, wind energy and other renewable energy source could be developed and used where feasible to exploit such energy sources. Moreover, mobile dairy plant suitable and flexible to pastoral and agro-pastoral settings could be introduced for sustainable development of the sector. Mobile plants can be run by solar energy and supply pasteurized camel milk and other dairy products to the market.

Tailor made trainings and awareness creation should be done on quality and quantity milk production, preservation, processing, and marketing of camel milk. Promotion of camel milk and dairy products consumption should be promoted through mass median, school milk day and other relevant media. Establishment of dairy cooperatives have indispensable role for development of camel dairy sector and hence deserves to get priority and protection from the government. With out dairy cooperatives it will be very difficult to bring the desired development and promotion of camel dairy in particular and dairy in general.

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Appendix

Table 7. Description of starter cultures

Culture	Description	Taxonomy
STI-12	Homofermentative thermophilic culture	*Streptococcus thermophilus*
RST-743	Blended of mesophilic and thermophilic homofermentative culture	*Lactococcus lactis subsp. lactis* and *Streptococcus thermophilus*
R-707	Mesophilic homofermentative	*Lactococcus lactis* subsp. lactis, *Lactococcus lactis* subsp. cremoris
XPL-2	Blended of mesophilic aromatic LD and thermophilic cultures	*Lactococcus lactis* subsp. *cremoris*, *Lactococcus lactis* subsp. *lactis*, *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis*, *Leuconostoc* species, and *Streptococcus thermophilus.*
CHN-22	Mesophilic aromatic LD culture (produce flavour and CO_2)	*Lactococcus lactis* subsp. *cremoris*, *Leuconostoc pseudomesenteroides*, *Lactococcus lactis* subsp. *lactis* biovar *diacetylactis*, *Lactococcus lactis* subsp. *lactis*, *Leuconostoc mesenteroides*.
Yoflex mild 1.0	Thermophilic yoghurt culture	*Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus thermophilus*
YF-L904	Thermophilic yoghurt culture	*Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus thermophilus*

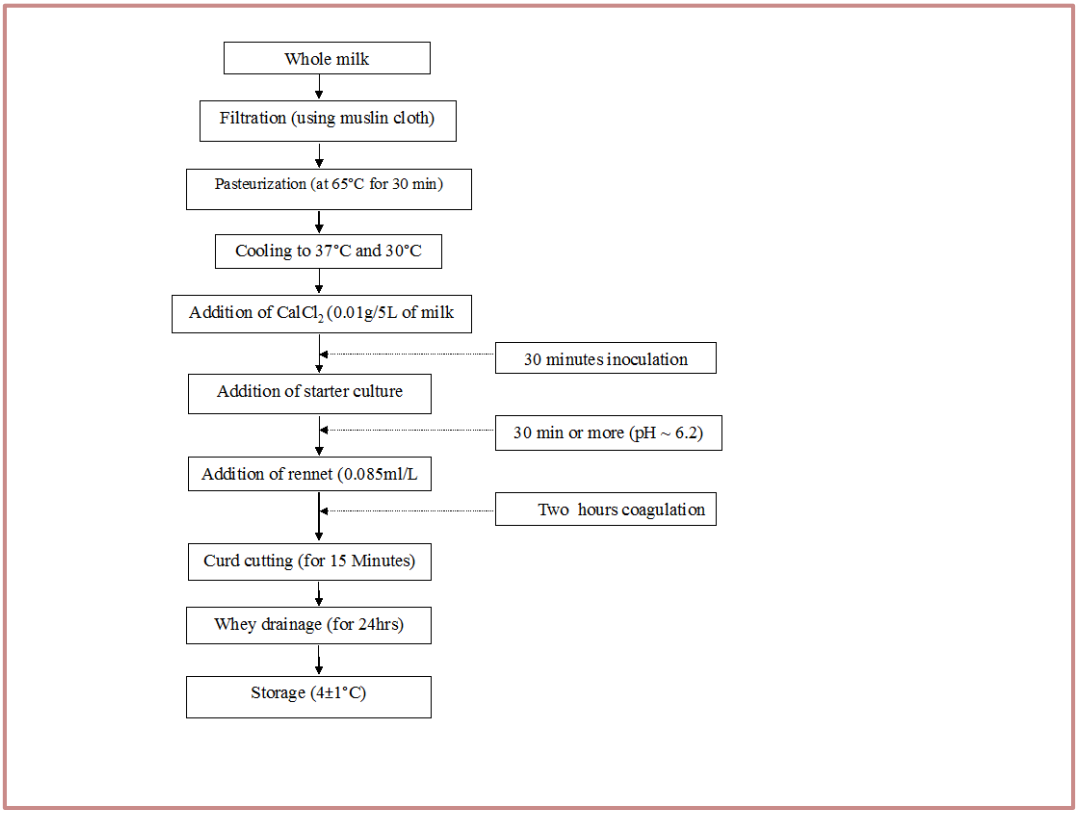

Figure 7. Flow chart for the manufacture of soft white cheese (SWC) (Source: Birhanu Bekele et al., 2019

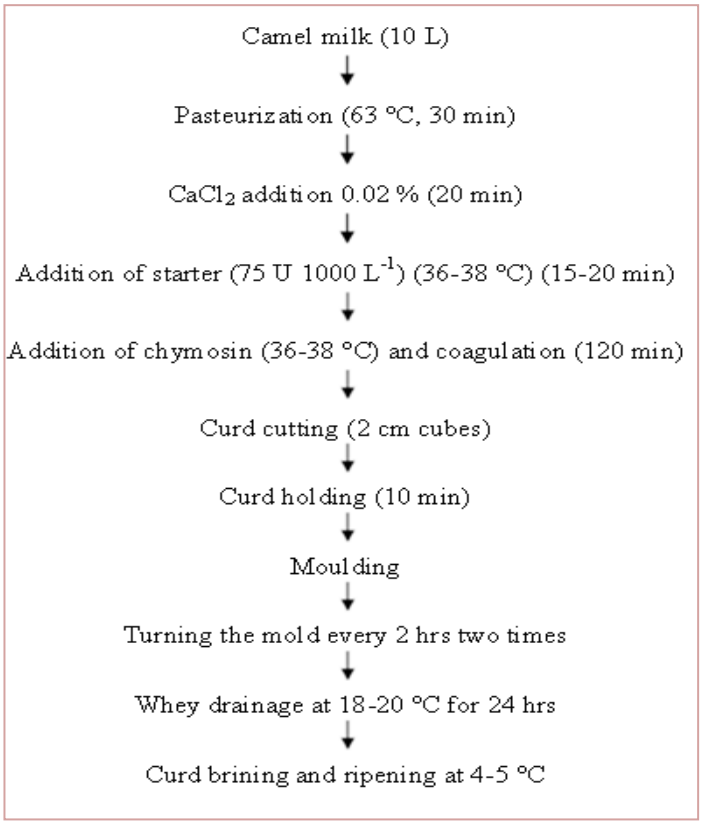

Figure 8. Model cheese recipe for soft brined cheese made from camel milk

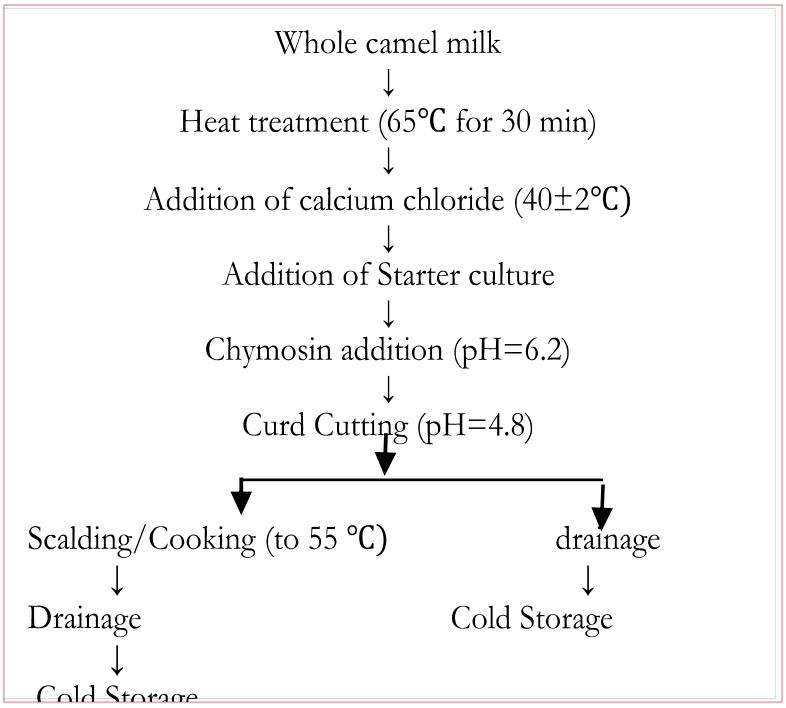

Figure 9. Flow diagram adopted for manufacture of soft unripen cheese from camel milk (Mehaia, 2006)

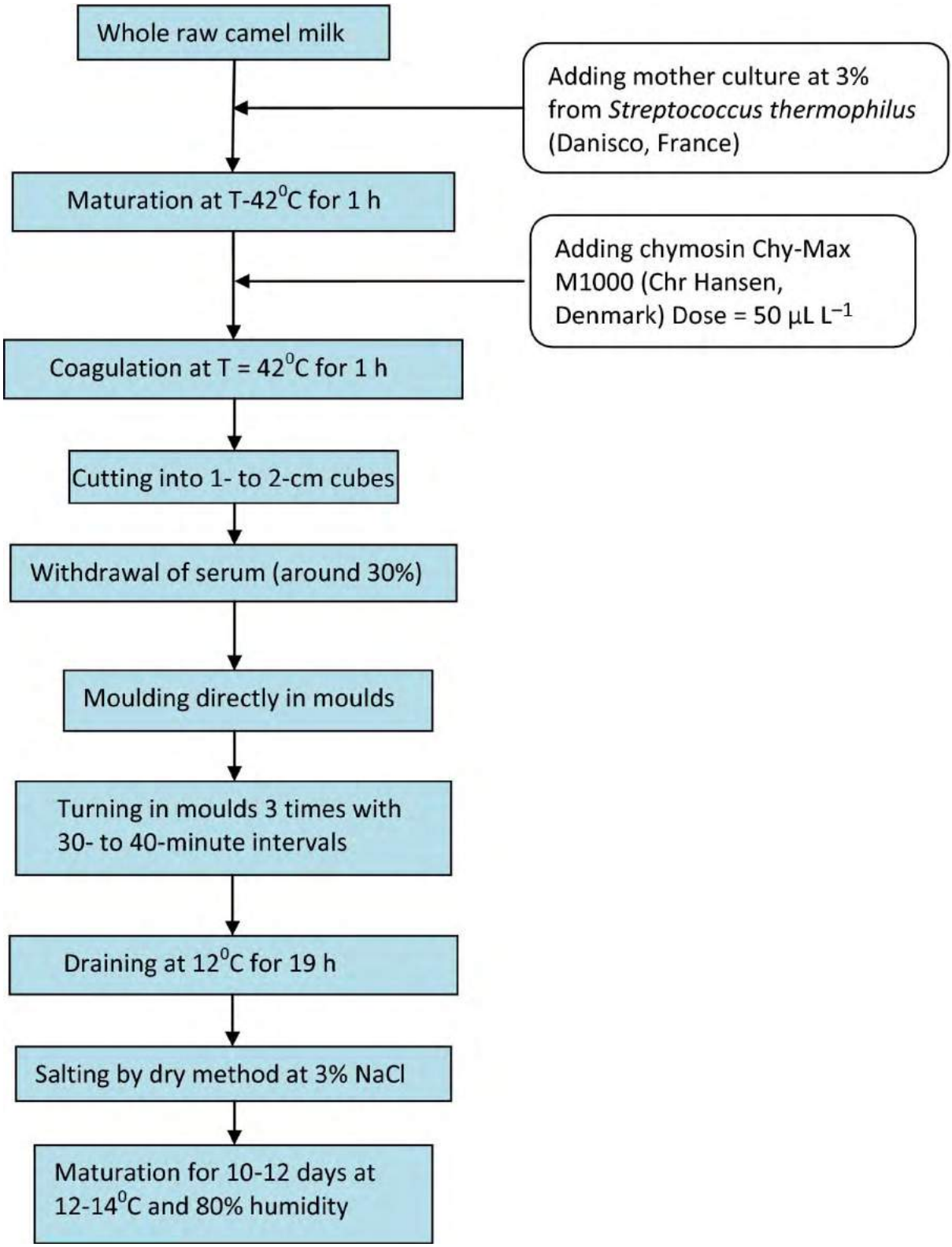

Figure 10. Process flow diagram for brine salted cheese (Feta type) made with camel milk

Average Estimates of Genetic and Phenotypic Correlations among Production and Reproduction Traits in Goats

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Abstract

A meta-analysis of 84 published reports on goats was conducted to calculate weighted and unweighted average genetic (r_g) and phenotypic (r_p) correlations for growth, reproduction and milk production traits. Weighted averages of r_p and r_g among growth traits ranged from -0.06 to 0.84 and 0.01 to 0.98, respectively. Weighted average r_p among milk traits ranged from 0.18 to 0.94. In most cases, average r_g had higher observed standard deviations compared to the theoretical standard error. For the r_p, local estimates should be used instead of averages presented here due to the fact that r_p was estimated with larger theoretical standard errors than mean standard errors. The average estimates of parameters calculated in the present study could be applied for a wide range of conditions and could complement global goat genetic improvement initiatives. However, it should be noted that since most of the traits reviewed here were not frequently studied, further genetic parameter estimations for growth, reproduction, and milk traits of goat are required.

Key words: Correlation; goat; unweighted averages; weighted averages;

Introduction

Goats significantly contribute to the national economy and livelihood of the poor in many developing countries (Peacock, 2005; Aziz, 2010). However, their productivity is often low as a result of many interrelated factors including the genetic potential of the indigenous stock. Planning and implementing sustainable breeding programs has the potential to contribute to bridging the performance gap. Genetic parameters including genetic correlations and heritabilities are required for planning breeding strategies and genetic evaluation programs in livestock (Willam et al., 2008).

In quantitative genetics it is commonly believed that the genetic parameters refer to the population in which they are estimated. However, sufficient time and suitable data are limiting factors of the parameter estimations available for specific populations (Koots et al., 1994a, b). Even when parameter estimates are available, precision is generally low. On the other hand, Koots and Gibson (1996) indicated that referring estimates of genetic parameters to the population in which they are estimated should not be a universally accepted approach. Values estimated elsewhere could be used for populations or breeds with no genetic correlation estimates. Hence, the genetic parameters including genetic correlations might be more accurately estimated by pooling results from literature and combining them with specific population estimates (Koots et al., 1994a, b; Koots and Gibson, 1996; Lobo et al., 2000), where available.

There are different ways to pool and present genetic parameter estimated from literature. For instance, Cammack et al. (2009) summarized in the form of ranges; Utrera and Vleck (2004) presented in the

unweighted form; Safari et al. (2005) presented in the weighted form; Koots et al. (1994a, b) and Lobo et al. (2000) presented both unweighted and weighted forms.

While literature estimates of genetic parameters (correlations) are available for cattle (Koots et al., 1994a; Koots et al., 1994b; Lobo et al., 2000; Utrera and Vleck, 2004; Cammack et al., 2009) and sheep (Safari et al., 2005), such information are lacking for goat traits. Therefore, this review aims at filling these gaps and contributing to the global goat genetic improvement initiatives. While the need for genetic parameters in goats was noticed during the implementation of a community based breeding program of goats in Ethiopia, the genetic correlation estimates pesented here could target global application. The specific objectives were to present unweighted and weighted average correlations for growth, reproduction and milk production traits in goats based on meta-analysis of published literature.

Materials and Methods

Construction of the dataset: choice of papers and traits studied

A data set of genetic correlation for growth, reproduction and milk production traits was constructed from 84 independent publications. The following criteria were used for including estimates from a paper in this study: where the paper (1) presents estimates informative descriptions available and (2) reports standard errors for heritability and/or the number of observations. Six hundered seventy eight correlation estimates were used in calculating reliable genetic and phenotypic correlations in goats. The number of papers by country and citations by breed are presented in Tables 1 and 2, respectively. Traits related to growth, fertility, milk production, survival and productivity which have practical importance for goat breeding programs were included in the study (Table 3).

Average genetic parameters

Unweighted and weighted average parameters included phenotypic (r_p) and genetic (r_g) correlations. Unweighted averages across publications were obtained directly with SD, where applicable. For both r_p and genetic r_g, average of weighted standard errors and observed standard deviations were provided.

Table 1. Number of reports by countries

Number of reports	Countries
10	Iran
8	India
5	Bangladesh, China, Nigeria, South Africa, Thailand
3	Ethiopia, France, United States of America
2	Brazil, Croatia, Italy, Mexico, New Zealand, Saudi Arabia, Serbia, Sudan
1	Arab Emirate, Caribbean, Gambia, Indonesia, Iraq, Japan, Mediterranean basin, Morocco, Norway, Pakistan, Poland, Ruanda, Slovenia, Syria, Tanzania, Turkey
84	Total reports

Table 2. Papers cited by breed

Breed	References
Alpine	Bélichon et al., 1998, Brito et al., 2011, Brenik et al., 2000, García-peniche et al.2010, Kasap et al.,2012, Kantanamalakul et al., 2010, McManus et al., 2008, Montaldo et al., 2012 and Mourad and Anous, 1998
Saanen	Brenik et al., 2000, Brito et al., 2011, Bélichon et al., 1998, Ishag et al., 2012, Kantanamalakul et al., 2008, Kasap et al., 2013, Kosum et al., 2004, McManus et al., 2008, Montaldo et al., 2010, Morris et al., 2011, Supakornand Pralomkarn, 2009, Supakorn and Pralomkarn, 2012, Thepparat et al., 2012, Torres-Vázquez et al., 2009 and Valencia et al., 2007
Angora	Snyman and Olivier, 1996 and Snyman, 2012,
Anglo-Nubian	Kantanamalakul et al., 2008, Supakornand Pralomkarn, 2009, Supakorn and Pralomkarn, 2012 and Thepparat et al., 2012,
Thai-Native	Anothaisinthawee et al., 2012, Kantanamalakul et al., 2008, Supakornand Pralomkarn, 2009, Supakorn and Pralomkarn, 2012 and Thepparat et al., 2012
Arsi-Bale	Bedhane et al., 2012 and Bedhane et al., 2013
Black-Bengal	Faruque et al., 2010, Mia et al., 2013a, Mia et al., 2013b, Mia et al., 2014
Boar	Hongping et al., 2002, Nieker, 1996, Schoeman et al., 1997, Zhang et al., 2008, Zhang et al., 2009a, Zhang et al., 2009b,
Creole	Gunia et al., 2011
Draa	Boujenane and Hazzab, 2008
Iranian indigenous	Shamshirgaran and Tahmoorespur, 2012
Kotchi	Yadav et al., 2004 and Yadav et al., 2009
Markhoz	Rashidi et al., 2008, Rashidi et al., 2011 and Rashidi et al., 2015
Nubian	García-peniche et al., 2011 and Montaldo et al., 2010
Raeini	Barazandeh et al., 2012a, Barazandeh et al., 2012b, Gholizadeh et al., 2010 and Mohammadi et al., 2012
Toggenburg	García-peniche et al., 2012, McManus et al., 2008 and Montaldo et al., 2010
Jamunapari	Roy et al., 2008, Singh et al., 2009a and Singh et al., 2009b
LaMancha	Montaldo et al., 2010 and García-peniche et al., 2012
Zaraebi	Hamed et al., 2009, Osman, 2013, Shaat et al., 2007 and Shaat and Maki-Tanila, 2009
West African Dwarf	Bosso et al., 2007, Odubote, 1996 and Otuma and Onu, 2013
Adani	Dashtizadeh et al., 2012
Naeini	Baneh et al., 2012
Aradi	AL- Saef and Mousa, 2013 and Al-Saef, 2013
Norwegian	Bagnicka et al., 2007
Polish	Bagnicka et al., 2007

Breed	References
Balkan	Petrović et al., 2012
Oberhasli	García-peniche et al., 2011
Blended	Rege and Shibre, 1994
Emirate	Al-Shorepy et al., 2002
New Zeland Cashmere	Baker et al., 1991
Red Sokoto	Ishag et al., 2012
Ettawa Grade	Hasan et al., 2014
Exotic	Hassan et al., 2013
Sahelian	Otuma and Osakwe, 2008
German fawn	Ćinkulov et al., 2006
Sirohi	Gowane et al., 2011
Iraqi local	Hermiz et al., 2009
Jakhrana	Mandal et al., 2010
Sicilian Girgentana	Portolano et al., 2002
Long leg goat	Otuma and Onu, 2013
Local (Goat)	Alade et al., 2010
Sudanese Nubian	Ballal et al., 2008
Maltese	Delfino et al., 2011
Marwari	Raj et al., 2001
Matabele	Assan et al., 2011
Mediterranean	Mavrogenis, 1988
US Dairy goat	Castañeda-Bustos et al., 2014
Teddy	Tahir et al., 1995
Common West African Dwarf	Kantanamalakul et al., 2010, Mouradand Anous, 1998

Table 3. List of traits included in the study (with abbreviations)

Weight traits	birth weight (bwt), 20 (20d) and 70 (70d) days weights, one (1mw), two (2mw), three (3mw), four (4mw), five (5mw), six (6mw), seven (7mw), eight (8mw), nine (9mw), 11 (11mw), 12 (12mw), 16 (16mw) and 18 (18mw) month weights
Growth traits	average daily gains during pre-weaning (ADG1), daily gain from three to six months (ADG2), six to 12 months (ADG3)
Reproduction traits	kidding interval (KI), 1st kidding interval (1st KI), litter size at birth (LSB), litter size at weaning (LSW), litter weight at birth (LWB), gestation length (GL) and age at first kidding (AFK).
Efficiency traits	Kleiber Ratio (KR)
Survival rate	survival to weaning
Milk production traits	average daily yield (ADM), ninety days (90DMY), five months (150MY), nine months (270MY) first lactation (1stLMY) and lactation (LMY) milk yields; protein (PROTY) yield, fat (FATY) yield, protein percentage (P%), fat percentage (F%), combined protein and fat (COMB P&F), ratio of protein to fat (Ratio P:F) and lactation lengths (LL).

Weighted average correlations

Weighted average phenotypic and genetic correlations were transformed to an approximate normal scale using Fisher's Z transformation as follows (r= correlation value): $Z = 0.5log\left[\frac{r+1}{r-1}\right]$

The standard error (SE_Z) of Z was obtained using the following equation (n= number of records for phenotypic correlations (Safari et al., 2005), and number of sires for the genetic correlations (Koots et al., 1994b) $SE_Z = [n-3]^{-1/2}$. Then values for Z were pooled over studies by weighing with the inverse of their sampling variance as follows: $Z_{pooled} = \frac{\sum_{i=1}^{n} z_i/(SE_{Z_i})^2}{\sum_{i=1}^{n} 1/(SE_{Z_i})^2}$.

The mean pooled Z values were then transformed back to correlations as follows: $r = \frac{((e^{2Z})-1)}{((e^{2Z})+1)}$.

Results and Discussion

Estimated average correlations

Unweighted average correlations

Unweighted average estimates of phenotypic (r_p) and genetic (r_g) correlations among growth traits (Table 4) and between reproduction, milk production traits, between reproduction and milk production traits and between litter size at birth and growth traits (Table 5) were calculated. Unweighted average r_p among growth traits ranged from -0.25 (6mw, ADG3) to 0.95 (5mw, 6mw), whereas the unweighted average r_g ranged from -0.20 (3mw, ADG2) to 0.98 (4mw, 5mw) among growth traits. Unweighted average r_p among reproduction traits were in between zero and 0.24 except for (LSB, GP) which was as high as 0.54. The unweighted average r_g among reproduction traits were a bit higher than unweighted average r_p ranging from 0.34 (LWW, AFK) to 0.79 (LSB, LSW). The unweighted r_p and r_g among milk production traits ranged from 0.14 (150MY, LL) to 0.95 (90MY, 150MY) and 0.36 (90MY, LL) to 0.94 (DMY, LMY), respectively.

The correlation values tended to be higher for age-adjacent traits. For instances, the unweighted r_g value between bwt and 3mw was higher (0.55) compared to the unweighted r_g between bwt and 12mw (0.33). The trend was also the same in the case of weighed r_g. This could be because genes affecting adjacent age traits are likely more similar than genes affecting distant age traits as genes have switch-off and switch-on times. Hence, pre-weaning growth traits have less potential to predict post weaning growth traits. In the same fashion, early life growth traits had smaller unweighted average r_p with the latter age weights. These findings are in agreement with Koots et al. (1994b) and Lobo et al. (2000).

For 57% of the unweighted average r_p and r_g no SD could be calculated as they were contributed by single studies. In the reports of Koots et al. (1994b), this figure was about 60%. In about 30% of the unweighted average values, two to ten studies contributed to the estimates while the remaining estimates were contributed by more than 10 studies. In most cases the unweighted averages of r_p were smaller than unweighted average r_g.

Table 4. Unweighted phenotypic (r_p) and genetic (r_p) correlations among growth traits with standard deviations (SD) where applicable

*Trait 1	Trait 2	r_p	r_g	Trait 1	Trait 2	r_p	r_g
Bwt	Pre	0.17±0.139b	0.33±0.258c	2mw	3mw	0.86±0.108c	0.85±0.138c
bwt	1mw	0.43±0.132c	0.90±0.119c	2mw	4mw	0.76±0.078c	0.73±0.099c
bwt	2mw	0.59±0.226c	0.74±0.247c	2mw	5mw	0.66±0.085c	0.66±0.148c
bwt	3mw	0.35±0.197a	0.55±0.171a	2mw	6mw	0.64±0.067c	0.74±0.140c
bwt	4mw	0.29±0.121c	0.63±0.196c	2mw	7mw	0.59	0.54
bwt	5mw	0.31±0.087c	0.44±0.096c	2mw	ADG2	0.03	0.13± 0.157
bwt	6mw	0.32±0.183b	0.37±0.206b	3mw	4mw	0.87±0.106c	0.86±0.163c
Bwt	ADG2	-	0.18±0.151c	3mw	5mw	0.63±0.283c	0.88±0.149c
bwt	7mw	0.30	0.50±0.269c	3mw	6mw	0.67±0.133b	0.72±0.238b
bwt	8mw	0.25	0.29	3mw	ADG2	-0.01±0.044c	-0.01
bwt	9mw	0.35±0.192b	0.31±0.165c	3mw	7mw	0.69	0.64
bwt	12mw	0.26±0.167b	0.33±0.251b	3mw	8mw	0.60	-0.02
Bwt	ADG3	-	-0.02	3mw	9mw	0.58±0.149b	0.62±0.229b
bwt	16mw	0.24	0.57	3mw	ADG3		0.61
bwt	18mw	0.23	0.15	3mw	12mw	0.54±0.136b	0.52±0.217b
Bwt	KR	-	0.25±0.286c	3mw	16mw	0.54	0.03
20d	70d	0.12	0.17	3mw	18mw	0.64	0.64
20d	3mw	0.10	0.14	3mw	KR	-	0.95±0.040c
20d	5mw	0.11	0.14	4mw	5mw	0.94±0.042c	0.98±0.014c
20d	6mw	0.10	0.14	4mw	6mw	0.85±0.127c	0.95±0.028c
20d	9mw	0.09	0.11	4mw	7mw	0.98	0.89
20d	12mw	0.09	0.10	5mw	6mw	0.95±0.049c	0.95±0.055c
20d	3mw	0.19	0.99	5mw	7mw	0.97	0.94
1mw	2mw	0.81±0.095c	0.86±0.150c	5mw	9mw	0.77	0.73
1mw	3mw	0.59±0.68c	0.83±0.188c	5mw	12mw	0.71	0.64
1mw	4mw	0.63±0.08c	0.56±0.0.382c	5mw	DAG2	0.80	0.93
1mw	5mw	0.56±0.11c	0.55±0.304c	6mw	ADG2	0.74	0.54±0.354c
1mw	6mw	0.40±0.19c	0.45±0.340c	6mw	7mw	0.98	0.88
1mw	7mw	0.55	0.54	6mw	9mw	0.80±0.069b	0.71±0.270c
70d	5mw	0.26	0.93	6mw	ADG3	-0.25	0.64
70d	6mw	0.25	0.90	6mw	12mw	0.70±0.077b	0.68±0.216b
70d	9mw	0.19	0.51	6mw	18mw	0.74	0.74
70d	12mw	0.19	0.55	8mw	12mw	0.83	0.97
Pre	2mw	0.85	0.99	8mw	16mw	0.76	0.94
Pre	3mw	0.81±0.245c	0.87±0.189c	9mw	12mw	0.83±0.079c	0.86±0.119c
Pre	5mw	0.58	0.49	9mw	ADG2	0.49	0.71
Pre	6mw	0.47±0.254c	0.58±0.233	9mw	ADG3	0.13	0.65
Pre	ADG2	0.24±0.503c	-	12mw	ADG2	0.41±0.127c	0.50±0.332c
Pre	9mw	0.63±0.007c	0.67±0.035	12mw	ADG3	-0.003	-
Pre	ADG3	-0.28	-	12mw	Post	0.32	0.26
Pre	KR	0.88	0.96±0.040c				

*bwt=birth weight; 20d and 70d are weights at 20 and 70 days of age, respectively; 1mw=weight at one month of age; 2mw, 3mw, 4mw, 5mw, 6mw, 7mw, 8mw, 9mw, 11mw, 12mw, 16mw, 18mw are, in respective order, weights at two, three, four, five, six, seven, eight, nine, 11, 12, 16 and 18 months of age; KR= kleiber ratio; pre, ADG2, and ADG3 are average daily gains during pre-weaning, three to six month and six to 12 months of ages, respectively; c=estimates were from less than 10 studies, b=estimates were from 10 – 20 studies, a=estimates were from more than 20 studies. If values are not given is parenthesis, then the standard errors of the genetic correlation were not reported in a study (ies), *=values without standard deviation were sourced from single study.*

Table 5. Unweighted phenotypic (r_p) and genetic (r_g) correlations between milk production and reproductive traits in goat

Trait 1	Trait 2	r_p	r_g	Trait 1	Trait 2	r_p	r_g
Among reproduction traits				Between reproduction and milk traits			
LSB	GP	0.54	0.48	LSB	LMY	0.25	0.08
LSB	LSW	0.24	0.79±0.131c	AFK	LMY	0.04	-0.18
LSB	LWB	0.21	-	AFK	PROTY	0.05	-0.17
LSB	LWW	0.21	-	AFK	FATY	0.08	-0.09
LWB	KI	-	0.53	KI	LL	0.35±0.704c	
LSB	AFK	0.15	0.61	Among milk production traits			
LSB	KI	-0.06	0.69	DMY	90MY	0.75±0.048c	0.91±0.137
LSW	AFK	0.11	0.34	DMY	150MY	0.87	-
LSW	KI	-	0.59	DMY	LMY	0.47±0.573c	0.94±0.115c
LWW	KI	-0.03		90MY	150MY	0.95±0.057c	-
LWB	AFK	0.09	0.61	90MY	LMY	0.81±0.013c	-
LWW	AFK	0.05	0.39	90MY	LL	0.16±0.042c	0.36±0.702
Between reproduction and growth traits				150MY	LMY	0.93	-
LSB	Bwt	0.30	-0.11±0.320c	150MY	LL	0.14±0c	-
LSB	Pre	-0.07	-0.22±0.141c	LMY	PROTY	0.94.016c	0.89±0.025c
LSB	9mw	-0.20	-0.12	LMY	FATY	0.85±0.010c	0.75±0.026c
LSB	12mw	-0.44	-0.50	LMY	LL	0.50±0.194c	0.50±0.438c
LSB	ADG2		-0.15±0.099c	PROTY	FATY	0.88c	0.83

DMY, 90MY, 150MY, LMY, and LL, are average daily, 90 days, 150 days, lactation milk yields and lactation length, respectively; PROTY and FATY, are protein yield, fat yield, in respective order; LSB, LSW, LWB, LWW, KI, AFK, are litter size at birth, litter size at weaning, litter weight at birth, litter weight at weaning, kidding interval, first kidding interval, respectively; bwt, pre, 9mw, 12mw and post are birth weight, pre-weaning weight, 9 months weight and 12 months weight, respectively; c=estimates were from less than 10 studies.

Weighted average correlations

Weighted average r_p and r_g among growth and reproduction traits, were given in Tables 6 and 7. The range of weighted average r_p and r_g among growth traits were from -0.06 to 0.84 and from 0.01 to 0.94, respectively. The weighted average r_p among milk production traits ranged from 0.18 to 0.94 whereas weighted average r_g among milk production traits were only available in few studies. Weighted averages r_p and r_g among reproduction traits were not presented due to lack of number of records required to calculate r_p and number of sires required to calculate r_g in the reviewed papers for these traits.

The number of studies contributing to weighted average correlations was smaller than for unweighted average correlations because of two facts: (1) correlation estimates sourced from single studies were not included as the Fisher's transformation returns the same value both for unweighted and weighted correlations; (2) where the number of sires for r_g or records for r_p were not indicated in the methodology of the study, weighted average correlations were not estimated. Additionally, correlation values less or equal to −1.00 or greater or equal to +1.00 such as those in Barazandeh et al. (2012) were also ignored from the calculation of weighted average correlations. Except for the weighted average r_p between 3mw and ADG2, all weighted average correlations were positive.

Overall, weighted genetic correlations among growth traits ranged from moderate to high, indicating the possibility of indirect selection among these traits. However, the genetic correlation between bwt

and subsequent weights declined after three months of age, which means bwt may not be a trait of choice for indirect improvement of weights at a later age like 6mw, 9mw or 12mw. Instead, 3mw to 6mw growth traits could be targeted for direct or indirect genetic improvement.

Observed standard deviations and theoretical standard errors

Observed standard deviation of phenotypic (PSD) and genetic (GSD) and predicted standard error of phenotypic (PPSE) and genetic (GPSE) correlations are presented in Table 8. The combinations of traits presented were those for which number of studies was greater or equal to seven for the calculation of PSE. Mean of observed standard deviation for a correlation is direct average of standard deviations of a correlation calculated from different traits and mean of theoretical standard error is average of theoretical standard errors of a correlation calculated from different traits based on a formula. Comparison of the two parameters for a correlation could indicate the source (s) of variation (s). For instance, if the mean observed standard deviation is greater than mean of theoretical standard error, for a correlation, it could indicate that the presence of variation in true correlations, estimation error or both (Koots et al., 1994a). In the present study, mean of observed standard deviation of a correlation was found to be always higher than the mean of theoretical standard error (for r_p, 0.139 (PSD) versus 0.027 (PPES); for r_g, 0.218 (GSD) versus 0.136 (GPSE)). We investigated the effect variation of true correlations by fitting different exogenous factors including methods of parameter estimation and management levels of animals while estimating the parameter. We could not find sufficient evidence to indicate the presence of variation in true correlation. Koots and Gibson (1996) indicated that the variation between the observed standard deviation and theoretical error could be due to estimation errors. This and the former finding indicate that estimates are not as such influenced by exogenous factors including management levels of animals for parameter estimation, methods of estimation or breed encouraging the utility of the average estimated presented here as needed.

Table 6. Weighted mean genetic (above diagonal) and phenotypic (below diagonal) correlations among most frequently recorded growth traits in goat with number of studies in parenthesis.

Traits	bwt	3mw	6mw	9mw	12mw	Pre	ADG2
bwt		0.54(12)	0.39(8)	0.32(7)	0.32(8)	0.23(5)	0.02
3mw	0.36(14)		0.76(10)	0.63(8)	0.31(8)	0.98(4)	0.10
6mw	0.37(10)	0.28(12)		0.90(7)	0.74(7)	0.74	0.29
9mw	0.34(7)	0.61(8)	0.81(7)		0.89(7)	0.64(2)	0.59
12mw	0.27(8)	0.61(8)	0.74(8)	0.84(6)			0.26
Pre	0.13(5)	0.55(4)	0.65	0.63(2)	0.56		0.01(2)
ADG2	0.02	-0.06	0.30	0.13	0.32	0.04(2)	

bwt=birth weight; 3mw, 6mw, 9mw and 12mw are, in respective order, weights at three, six, nine and 12 months of age; pre and ADG2are average daily gains during pre-weaning and weaning to 6 months of age.

Table 7. Weighted mean phenotypic (r_p) and genetic (r_g) correlations among growth, milk and reproduction trait in goat with number of studies from which weighted mean was calculated in parenthesis.

Trait 1	Trait 2	r_p	r_g	Trait 1	Trait 2	r_p	r_g
Among growth traits				4mw	6mw	0.97	0.76
Bwt	1mw	0.48(3)	0.935(3)	5mw	6mw	0.99(2)	0.91
Bwt	2mw	0.74(3)	0.959(3	5mw	9mw	0.73	0.77
Bwt	KR	-0.001(2)	0.418(2)	Between reproduction and growth traits			
Bwt	4mw	0.16	0.740	LSB	Bwt	0.20(2)	
Bwt	5mw	0.21	0.400	LSB	Pre	-0.31(2)	-0.02(2)
Bwt	7mw	0.30	0.690	LSB	9mw	-0.12	
Bwt	16mw	0.24	0.570	LSB	12mw	-0.50	
1mw	2mw	0.84(2)	0.983(2)	LSB	ADG2	-0.09(2)	
1mw	3mw	0.59(2)	0.945(2)	Among milk production traits			
1mw	4mw	0.57	0.830	TDMY	90MY	0.73(2)	
1mw	5mw	0.48	0.760	TDMY	150MY	0.87(1)	
1mw	6mw	0.30(2)	0.521(2)	TDMY	LMY	0.25(2)	
2mw	3mw	0.85(3)	0.927(2)	TDMY	LL	0.77(2)	
2mw	4mw	0.74(2)	0.800	90MY	150MY	0.97(2)	
2mw	5mw	0.82	0.669(2)	90MY	LMY	0.78(4)	0.93(2)
2mw	6mw	0.82(2)	0.669(3)	90MY	LL	0.18(3)	
3mw	4mw	0.98	0.856(2)	150MY	LMY	0.93(2)	
3mw	5mw	0.97(2)	0.605(3)	150MY	LL	0.14(2)	
3mw	KR	0.98(2)	0.784(2)	LMY	PROTY	0.94(3)	
4mw	5mw	0.99	0.605(2)	LMY	LL	0.51(3)	0.52(2)

bwt=birth weight; 1mw=weight at one month of age; 2mw, 3mw, 4mw, 5mw, 6mw, 7mw, 9mw, 12mw, 16mw, 18mw are, in respective order, weights at two, three, four, five, six, seven, nine, 12, 16 and 18 months of age; KR= kleiber ratio; pre and post are average daily gains during pre-weaning and post-weaning, respectively.

Table 8. Comparison of observed standard deviation (SD) and theoretical standard error (PSE) of genetic correlations for some of trait combinations allowing comparison

Traits	PSE	PSD	PPSE	GSE	GSD	GPSE
Bwt, 3mw	0.023	0.197	0.028	0.206	0.171	0.144
Bwt, 6mw	0.020	0.183	0.028	0.27	0.206	0.029
Bwt, 9mw	0.010	0.192	0.032	0.225	0.165	0.174
Bwt, 12mw	0.016	0.167	0.027	0.090	0.251	0.168
3mw, 6mw	0.020	0.133	0.026	0.191	0.238	0.134
3mw, 9mw	0.025	0.149	0.030	0.131	0.229	0.163
3mw, 12mw	0.010	0.136	0.027	0.228	0.217	0.131
6mw, 9mw	0.015	0.069	0.022	0.062	0.270	0.127
6mw, 12mw	0.015	0.027	0.029	0.197	0.216	0.144
Average	0.017	0.139	0.027	0.178	0.218	0.136

PSE = mean of reported standard errors for phenotypic correlations, PSD = Observed standard deviation of phenotypic correlations, PPSE = theoretical standard error of phenotypic correlations, GSE = Mean of reported standard errors of genetic correlations, GSD= standard deviations of genetic correlations, GPSE=theoretical standard error of genetic correlations, Bwt=birth weight, 3mw= three month weight, 6mw=six month weight, 9mw=nine month weight, 12mw=12month weight

Conclusion

Based on our findings and those of others (Koots et al., 1994a, b; Lobo et al., 2000; Safari et al., 2005), weighted average r_g should be preferred to unweighted averages where information is lacking. The absence of significant differences for the tested fixed factors on parameter estimate in growth traits also reinforces reliability of the weighted averages of genetic and phenotypic correlations presented here. The average estimates should be combined with the local/specific estimates to plan goat breeding strategies and genetic evaluation programs. For the r_p, local estimates should be used instead of averages presented here; this is due to the fact that r_p was estimated with larger theoretical standard errors than mean standard errors. The average estimates of correlation parameters calculated in the present study could be applied for a wide range of conditions and could complement global goat genetic improvement initiatives. However, it should be noted that since most of the traits reviewed here were not frequently studied, further genetic parameter estimations for growth, reproduction, and milk traits of goat are required.

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Benchmark Parameters for Community Based Genetic Improvement of Abergelle, Central Highland and Woyto-Guji Indigenous goat breeds in Ethiopia

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Abstract

Assessments of production parameters and flock productivity were made in three indigenous goat breeds of Ethiopia. The goat breeds included Abergelle (AB), Central Highland (CH) and Woyto-Guji (WG). Objectives of this paper were (1) to estimate production parameters including three month weight (3mw), kidding intervals (KI) and litter size (LSB) at birth for AB, CH and WG and (2) to assess the productivity of the three indigenous goat breeds. As AB is used for milk production, adjustment was made to their 3mw. The overall mean of 3mw (kg) were 7.44, 10.96 and 9.38 for AB, CH and WG goat breeds, respectively. Generally, wet season, male sex and single birth resulted in higher 3mw for the three breeds. The overall means of KI were 362, 268 and 309 days for AB, CH and WG respectivel. The overall means of the LSB for the goat breeds, in respective order, were 1.03, 1.40 and 1.09 per doe per parturition. The flock productivity ranged from 0.27 to 0.53. Higher LSB, survival to three months (S3M), 3mw and number of parturition per year (N) resulted in higher flock productivity. CH goat breed had the highest flock productivity than the rest. The parameters estimated in this paper could be used as benchmark for the designed community based breeding program of goats in the studied localities.

Key words: Doe; growth; litter size; kidding interval; milk

Introduction

In increased human population, urbanization and changing climate, goat population in Ethiopia showed an increasing trend (FAO, 2014; CSA, 2017). The recent goat population estimate of Ethiopia puts it at around 30.20 million (CSA, 2017). In previous decades, the goat population estimate of the country was considerably smaller than the sheep population. However, since very recently, the ratio of goat to sheep showed an increasing trend; 0.93 (CSA, 2012), 0.99 (CSA, 2015) and 0.98 (CSA, 2017). This might be an indication that goats are becoming equally important as sheep in Ethiopia.

In developing countries, including Ethiopia, indigenous goats make valuable contributions, especially to the poor in the rural areas. They are important sources of meat, milk, manure, fibers & skins, and satisfy various cultural and religious functions (Tesfaye, 2004; Aziz, 2010; Devendra, 2012).

The importance of this valuable genetic resource is, however, underestimated and the contribution to the livelihood of the poor is inadequately understood (Kosgey and Okeyo, 2007; Aziz, 2010). The

productivity of these indigenous goats is also low as a result of many interrelated factors including lack of applicable and impactful breeding programs.

Genetic improvement through establishment of central nucleus small ruminant flocks in the research centres in Ethiopia was known to be ineffective due to various factors (Getachew et al., 2018). As an alternative, community-based breeding program (CBBP) of small ruminants has emerged. The CBBP is a design of breeding scheme that is deemed suitable for smallholder farming system (Gizaw et al., 2014). This approach is preferred to the more common top-down breeding programs that are mostly established on governmental stations in developing countries (Mueller et al., 2015b) and particularly suitable for small ruminants. The CBBPs have been established in different parts of the world; for sheep and goats in Ethiopia (Duguma et al., 2011; Haile et al., 2011; Abegaz et al., 2014), for goats in Mexico (Wurzinger et al., 2013) and in Iran (Mueller et al., 2015a). Many African countries are also establishing the CBBP for small ruminants.

Implementation of CBBP of three indigenous goats in Ethiopia was done by the leading role of Bioscience for eastern and central Africa and International livestock Research Institute (BecA-ILRI) in six villages (CBBP sites). The breeds included Abergelle kept in arid agro-pastoral, Central Highland inhabiting crop-livestock production system and Woyto-Guji from semi-arid agro-pastoral production systems (Tatek et al., 2016). The implemented CBBP on these goat breeds are being monitored by the national research systems and being implemented with technical backup from International Center for Agricultural Research in Dry Areas (ICARDA).

Alternative breeding programs to the current ones had been simulated to improve the breeding objective traits of the three indigenous goat breeds in there reproducing habitat (Temesgen, 2016). In order to evaluate the genetic progress to be realized, however, benchmark indicators were not well documented. The values presented for Woyto-Guji and Centeral Highland (Ambo site) by Zergaw et al. (2016) and for Abergelle and Central Highland (Gonder site) by Alubel (2015) were based on small data size which question its representativeness. Flock productivity was not considered in any of the former works. In addition, in Alubel (2016), early live weights of Abergelle goat breeds were not adjusted for the milk consumed by their producers that could have been converted to weight. Therefore, the present work was designed with the objective of setting benchmarks for community based breeding programs for Abergelle, Centeral Highland and Woyto-Guji indigenous goats in Ethiopia based on which realized genetic improvements could be compared later. Lack of benchmarks against which genetic progresses could be compared was acknowledged as a gap in some of the CBBP of sheep in Ethiopia.

Materials and methods

Description of the study sites

The study was conducted in six villages and on three indigenous goat breeds, two villages per breed, in Ethiopia. The goat breeds were Abergelle (AB), Centeral Highland (CH) and Woyto-Guji (WG). The villages for AB, CH and WG are located in Tigray and Amhara, Amhara and Oromia and in and SNNP's (Southern Nations, Nationalities, and People's) region, respectively. Specific villages were *Dingur* (Tigray region) and *Blaku* (Amahara region) for AB, *Waykaw* (Amahara region) *and Tatessa* (Oromia region) for CH and *Messale* and *Arkisha* (SNNP's) for WG. The location of these villages is detailed in Table 1. Study site identification was guided by the respective district agriculturalists.

Table 1. Latitude, longitude, altitude and rainfall of the study villages

Parameters	*Dingur*	*Blaku*	*Waykaw*	*Tatessa*	*Massale*	*Arkisha*
Latitude	13° 22´	12° 81´	12° 86´	9° 54´	5° 21´	5° 26´
Longitude	38° 89´	38°76´	37° 35´	38°23´	37° 26´	37° 34´
Altitude[#]	1731	1405	1192	2176	1383	1326
Rainfall (ml)*	711	547	1879	911	511	511

**=average rainfall of 2013 and 2014 (national meteorology agency of Ethiopia) and eteorology stations for rainfall were Abi Adi, Sekota, Tikil Dingay, Ambo Agriculture, and Konso, from left to right, respectively; #=meters above sea level .*

Recording and analyses of traits

In order to set the benchmarks for the anticipated community based breeding programs in AB, CH and WG, three production parameters and a flock productivity index were analyzed and presented. The production parameters included weight (kg) at three months (3mw), litter size (LSB) at birth and kidding interval (day) (KI). *Ad hoc* enumerators were hired to collect data on production of growth and reproduction traits. The enumerators were recording weight of kids at birth (birth weight), live weight at three months, live weight at six month, and post-partum weight right after birth. In this paper, the three month weights, survival rate to three months (S3M) and post partum weights were considered. The reproduction traits were kidding interval (KI) and litter size at birth (LSB). The types of births (whether kids were born single or twin) were captured at birth from which the type of births of kids was calculated whereas, the kidding intervals were derived from the already recorded data as the difference between consecutive parturitions for a doe. The data collection duration was from mid July 2013 to Mid April 2015 for all breeds.

On the other hand, a flock productivity index was computed based on various parameters generated from data specific to each breed (Table 6); these parameters included number of parturitions per year (N), LSB, S3M, 3mw, post partum weights. The overall mean values were considered while computing the flock productivity index. In addition, correction was made to 3mw of AB based on the information provided in Table 2.

In the analysis of all traits, fixed effects, of villages, year, season, type, sex, and parity of kids' birth were investigated. Parity of does was captured from owners at beginning of monitoring work of the base flock. Numbers of records were found to be unbalanced across year, type and parity of births. Records from triplets, parity ≥ seven and the year 2015 were small. Due to these reasons, merging of records in 2015 and 2014, from triplets and twins and from parity ≥ seven and parity six was made. In addition, post-partum weight of does was fitted as linear covariate for the analyses of 3mw where the rest were fitted as fixed effects.

Seasons were categorized into 'dry' and 'wet' based on 2013 and 2014 rain fall data accessed from the national meteorology agency of Ethiopia. Accordingly, 'wet' months were July, August and October in *Dingur*; July, August and September in *Blaku*; June, August, September, October and November in *Waykaw*; April – October in *Tatessa*; and January, March, June, August, September, October and November in *Massale* and *Arkisha*. The rest months in the respective villages were 'dry' season.

Productivity analysis

Using the estimated biological parameters generated from data specific to each breed, flock productivity analysis was made to investigate productivity at flock level. In analyzing the flock productivity, Bosman et al (1997) used parameters including flock weight which was not captured or hardly possible to capture in our cases. Due to this fact, flock mean weight was replaced by post partum weight (ppw) in the present study thinking that it could give good indication of the flock productivity. Flock productivity was assessed and compared across the three indigenous goat breeds using index given bellow:

$y = \frac{N \times LSB \times S3M \times 3mw}{PPWm}$ (Bosman et al 1997; Peacock 1987)

where y=productivity in kg live weight per kg post-partum weight per year; N = number of parturitions per year; LSB=litter size at birth; S3M = survival rate to three months of age; 3mw= live weight at three months (adjusted for milk consumed by producers for AB based on information given in Table 6); PPWm=mean postpartum weight of does.

Overall mean values or mean values of 3mw, LSB, KI and PPW were used in the calculation of this productivity index. Number of parturition per year was calculated based on overall mean KI values. When KI is less than 365 days, number of parturition is definitely more than one times and when the KI is more than 365 number of parturition per year is less than one times.

Milk was economically important trait in AB where producers compete for milk with kids (Alubel 2015; Tatek et al 2016); from CH and WG breeds, however, farmers do not milk goats. If this circumstance is not taken into account, flock productivity of AB would be underestimated. Therefore, the amount of milk consumed by producers which would otherwise be used by kids for growth was converted into growth based on information contained in Table 2.

Table 2. Metabolizable energy (ME) required per gram growth in kids (ME/ g growth), ME content of Abergelle goat milk and percentage milk consumed by producers

Parameters*	Values	Citations
ME/g growth	6.7	Temesgen (2016)
ME of AB goat milk (range)	881.75 (567.70 – 1306.63)	Muhi (unpublished data)
% of milk consumed by producers	50% (about milk from one teat)	Peacock, 1996

**Average daily milk yield was 453.38 ml and 308.10 ml in Dingur and Blaku villages, respectively (Temesgen 2016)*

Based on information contained in Table 2, kids at *Dingur* and *Blaku* were losing about 226.690 g and 154.050 g daily and these were about 199.880 kcal and 135.830 kcal, respectively. When converted to growth that was 29.830 g and 20.270 g for the villages which was, in respective order, 2.680 kg and 1.820 kg at three months of age, for the villages, hence these values were added on actual 3mw of AB goat breed at the two sites in order to favor them while assessing the flock productivity.

Results

Least squares means of weights at three months (3mw) are given in Table 3 for AB, CH and WG goat breeds. The overall mean of 3mw (kg) were 7.4, 11.0 and 9.4 for AB, CH and WG goat breeds, respectively. Generally, wet season, males and single born kids resulted in higher 3mw in the three breeds (Table 3). The 3mw showed an increment with a unit increment of the does' ppw in all the goat breeds (Table 3). The effect of parity of birth was not significant on 3mw of all the breeds. AB and WG kids born in 2013 had higher 3mw than those born in 2014. Contrary to AB, for CH kids, the vice versa was observed where kids born in 2014 had higher 3mw than those born in 2013.

Table 3. Least squares means ($\bar{X}$)± standard errors (SE) of three month weight (3mw) (kg) by fixed factors in three indigenous Ethiopian goat breeds under farmers' production practices

Factors¢		Abergelle		Central Highland		Woyto-Guji	
		N	$\bar{X}$±SE	N	$\bar{X}$±SE	N	$\bar{X}$±SE
Overall		885	7.4±1.41	779	11.0±2.30	504	9.4±1.44
Village¥			*p*=0.021		*p*=0.167		*p*< 0.001
	1	351	7.6±0.16a	376	10.6±0.16	199	7.4±0.14b
	2	534	7.3±0.18b	403	10.9±0.16	305	10.6±0.11a
Year			*p*< 0.001		*p*< 0.001		*p*< 0.001
	2013	539	7.9±0.17a	198	10.2±0.19b	157	9.3±0.14a
	2014	346	7.0±0.16b	581	11.4±0.11a	347	8.7±0.10b
Season			*p*=0.012		*p*=0.039		*p*=0.617
	Dry	829	7.2±0.14b	250	10.6±0.17b	243	9.0±0.12
	Wet	56	7.7±0.24a	529	11.0±0.11a	261	9.0±0.11
Sex			*p*=0.244		*p*=0.006		*p*=0.065
	Male	447	7.5±0.17	394	11.0±0.13a	280	9.1±0.11
	Female	438	7.4±0.17	385	10.6±0.14b	224	8.9±0.11
Birth type			*p*=0.276		*p*< 0.001		*p*=0.065
	Single	837	7.6±0.12	315	11.6±0.17a	419	9.2±0.08
	Twin	48	7.4±0.25	464	10.0±0.13b	85	8.8±0.17
	PPW	0.1±0.01		0.1±0.02		0.1±0.02	

N= number of observations; ¢= least squares means with different letter are significantly different; ¥=1=Dingur, Waykaw and Massale for AB, CH and WG breeds, respectively and 2=Blaku, Tatessa and Arkisha for AB, CH and WG, respectively; PPW=Post-partum weight.

The least squares means and standard errors of kidding intervals (KI), in days, are given in Table 4 for AB, CH and WG goat breeds. The overall means of KI were 362, 268 and 309 days for the breeds in respective order. Does that had their previous parturition in 2014 had shorter KI in AB and CH does. AB does having their previous parturition in *Blaku* had longer KI than does that had their previous parturition in *Dingur*. The KI of does for CH did not significantly differed by villages of production.

Table 4. Least squares means ($\bar{X}$)± standard errors (SE) of kidding intervals (days) in three indigenous Ethiopian goat breed under farmers' production practices

Factors[₡]	Abergelle		Central Highland		Woyto-Guji	
	N	$\bar{X}$±SE	N	$\bar{X}$±SE	N	$\bar{X}$±SE
Overall	229	362±82	162	268.1±72.21	59	309.5±89.42
Village[¥]		*p*< 0.001		*p*=0.116		*p*=0.109
1	98	304.8±21.08b	72	252.1±11.06	-	-
2	131	348.4±20.34a	90	276.2±9.31	-	-
Year		*p*< 0.001		*p*< 0.001		*p*=0.132
2013	203	371.9±18.55a	101	294.0±9.94	36	312.6±28.94
2014	26	281.3±24.29b	61	234.2±10.48	23	268.8±34.12

N= number of observations; ₡= least squares means with different letter are significantly different; ¥=1=Dingur, Waykaw and Massale for AB, CH and WG breeds, respectively and 2=Blaku, Tatessa and Arkisha for AB, CH and WG, respectively.

Least squares means of litter size at birth (LSB) are given in Table 5 for AB, CH, and WG goat breeds. The overall means of the LSB for the goat breeds, in respective order, were 1.00, 1.40 and 1.09 per doe per parturition. The CH does from *waykaw* were characterized with higher LSB than same breed does from Tatessa village. In similar fashion, CH does that had births during dry seasons had higher LSB than does that had births during wet seasons.

Table 5. Least squares means of litter size at birth (LSB) in Abergelle (AB), Central highland and Woyto-Guji (WG) breeds.

		AB	CH		WG	
Fixed factors[z]	N		N		N	
Overall	1159	1.00±0.170	714	1.40±0.450	601	1.09±0.290
Village[¥]		*p*=0.523		*p*< 0.001		*p*=0.105
1	541	1.03±0.008	290	1.56±0.030a	245	1.10±0.020
2	618	1.02±0.009	424	1.34±0.030b	356	1.14±0.020
Season		*p*=0.453		*p*=0.038		*p*=0.470
Dry	1009	1.03±0.005	216	1.49±0.030a	284	1.11±0.020
Wet	150	1.02±0.014	498	1.41±0.020b	317	1.13±0.020
Parity		*p*=0.005		*p*< 0.001		*p*< 0.001
1	247	1.00±0.012b	135	1.17±0.040b	146	1.02±0.020b
2	157	1.00±0.014b	136	1.25±0.040b	137	1.02±0.030b
3	204	1.03±0.013ab	135	1.45±0.040a	107	1.08±0.030b
4	223	1.03±0.012ab	124	1.60±0.040a	97	1.18±0.030a
5	190	1.05±0.013a	81	1.62±0.050a	63	1.18±0.030a
≥6	138	1.05±0.015a	103	1.60±0.050a	51	1.21±0.040a

N=number of observations (observations in LS3M were equal to observations in LSB in respective breeds and factors); z=least square means with different letters are significantly different. ¥=1=*Dingur*, *Waykaw* and *Massale* for AB, CH and WG breeds, respectively and 2=*Blaku*, *Tatessa* and *Arkisha* for AB, CH and WG, respectively.

Table 6. Summary of productivity parameters used in calculation of productivity indices in the three goat breeds**

Parameters*	Abergelle		Central Highland		Woyto-Guji	
	Dingur	*Blaku*	*Waykaw*	*Tatesa*	*Massale*	*Arkisha*
LSB	1.029	1.023	1.56	1.34	1.10	1.14
3mw(kg)	10.26	9.16	10.63	10.94	7.39	10.64
PPWm (kg)	24.35	24.44	35.37	29.83	28.01	25.37
N	1.20	1.05	1.45	1.32	1.18	1.18
S3M	0.628	0.888	0.785	0.785	0.777	0.777
Flock productivity	0.33	0.36	0.53	0.51	0.27	0.44

**Overall mean values were used; LSB= litter size at birth; 3mw= weight at three months of age and corrected for milk consumed by producers for AB breed; **=fitting LSB to logistic regression did not significantly improve the model than empty model and mean values were used; PPWm=mean values of post-partum weights; N=number of parturitions per year; S3M= survival rate to three months of age (Temesgen, 2016).*

Discussion

In the present study non-genetic factors influencing biological production traits including 3mw, LSB and KI were investigated for three indigenous goat breeds in Ethiopia. Using the estimated parameters as input, productivities at flock level was also studied. The effect of year and village of birth were significant on most of the production parameters. Those years and villages of birth, characterized by favorable conditions for feed production, had significantly better values that were in agreement with available literature (Hailu et al., 2005; Meza-Herrera et al., 2014; Ndlovu and Simela, 1996).

Three month weight

Generally, wet season, male sex and single birth resulted in higher 3mw in the present study in the three breeds. The present result was in agreement with various reports (Meza-Herrera, 2014; Hailu et al., 2005). In relation to endocrinal system, estrogen hormone has a limited effect on the growth of long bones in females and could be resulted in lighter body weight of females than males (Roshanfekr et al., 2011; Rashidi et al., 2008). Environmental conditions like temperature, humidity and rains known to have positive influence on live weights (Hailu et al., 2005; Ndlovu and Simela, 1996) might have been more favorable in the villages, seasons and year with superior 3mw.

Kidding Interval

Year of previous parturition in AB and CH does and village of previous parturition in the AB had significant influence on the KI. The present values of KI for CH and WG were in agreement with values reported by Ndlovu and Simela (1996) for east African goat, Đuričić et al. (2012) for Boer goat. The KI of the CH and WG goat breeds were shorter than reports of Marai et al. (2002). However, KI of AB breed were longer than the KI values in these report. Availability of feeds has direct influence on ovulation rate and fertility, since the nutritional stress appears to be a prime probable cause of long kidding interval in goats (Bushara et al., 2013). Differences in KI could also be attributed to differences in genetic makeup and managements (Gbangboche et al., 2006) as well.

Litter size

In agreement with this finding, parity of birth affected LSB of kids in Red Sokoto (Awemu et al., 1999) where LSB from mid parities were higher than the other parities. However, the values reported in the present study were lower than the values reported (1.57 - 1.77) by Meza-Herrera et al. (2014). In general, LSB is largely influenced by ovulation rate which was in turn substantially controlled by genotype and environment and can be increased by the pre-mating nutrition management in the case of ewes (Mukasa-Mugerwa and Lahlou-Kassi, 1995) which may also hold true in does.

Goat flock productivity

The flock productivity values in the present study were higher than the flock productivity from Nigerian goat studied by Bosman et al. (1997) that ranged from 0.19 – 0.22 kg. The variation in the productivity indices, generally, could be attributed to the values of the parameters composing the calculation of productivity indices. Higher N, LSB, S3M and 3mw resulted in higher flock productivity and the vice versa. As the result, CH goat breed had the highest productivity. The moderate flock productivity of the AB was due to the correction made to the three-month weight by assuming the conversion of milk consumed by households to the live weight of kid.

Conclusions

Single born and male kids had higher 3mw; locations with wet months of birth and does with higher postpartum weight also resulted in higher 3mw in AB, CH and WG indigenous goat breeds in Ethiopia. In general, CH goat breed was found to be the most productive when assessed with the help of flock productivity index. The higher productivity index value for CH breed was associated with higher LSB, S3M, N and 3mw. KI in AB breed was longest compared to the other two breeds due to harsh environments not favoring fastest onset of subsequent parturitions. Improvements in the production traits and then productivity at flock level could be attained by minimizing the effects of environmental sources. When comparison of productivity at flock level is to be made between breeds of multipurpose like AB, correction should be made to growth of kids by considering the amount of milk consumed by owners, which would have been consumed by kids. The parameters estimated in this paper could be used as benchmark for the anticipated CBBP of goats in the localities.

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Doe Productivity Evaluations of Abergelle, Centeral Highland and Woyto-Guji Indigenous Goat Breeds in Ethiopia

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Abstract

In line with the global population trend of the species, the goat number in Ethiopia had shown an increasing trend over recent years. With the help of SIDA (Swedish) funded project, Community Based Breeding Program (CBBP) was implemented on three indigenous goat breeds of Ethiopia, namely Abergelle (AB), Central Highland (CH) and Woyto-Guji (WG). The objective of this paper was to assess doe productivity based on kids' total live weight at three months (LWW), kids' survival rates to three months (S3M) and average daily milk yield (ADM). The LWW were also standardized by annum and post partum weight (ppw) of does and factors affecting these parameters were investigated. Five parameters, which could indicate the performance of does, including LWW, DMY, S3M, productivity expressed as the body weight of three months old kids produced per doe per year (index I) and per kg ppw per year (Index II) were investigated. The overall least squares mean of LWW for AB, CH and WG were 7.6, 15.3 and 10.2 kg, respectively. The overall mean of the DMY for AB was 367.10±139.79 ml. The S3M were 76.30, 78.50% and 77.70%, for AB, CH and WG, respectively. Overall means of index I and index II were 14.7 kg and 0.50, respectively. The highest index I and index II were from CH followed by WG. The year of birth significantly ($p<0.05$) affected LWW and S3M of AB, CH and WG, index I and index II. The ADM and S3M of AB and LWW of WG were significantly ($p<0.0001$) affected by village of births. The LWW and S3M of the three breeds were not significantly ($p>0.05$) influenced by sex of lambs. On the other hand, parities of births had significant influence ($p<0.05$) on DMY and S3M of AB and index I. Season of birth had also significant effect ($p<0.0001$) on LWW of AB only. The CH does produced about double LWW of AB does and about 150% LWW of WG does. The CH also had the highest productivity indices (index I and index II) followed by WG does. The kids from the CH had the highest survival rates. On the other hand, the AB does best suited for dual services (producing kids for meat and production of milk). Hence, goat meat investment priorities in Ethiopia could target Central Highland goats. In general, however, the productivity of the indigenous goat breeds should be optimized in their respective localities by minimizing possible factors hampering their productivity.

Keywords:- goat; indices; milk; productivity; survival; total kid weight.

Introduction

Ethiopia is believed to have the largest ruminant population in Africa; the country owns 60.4 million cattle, 31.3 million sheep and 32.7 million goats (CSA, 2018). The annual goats' population reports of Ethiopia were considerably smaller than the sheep population of the country. However, since very recently, the ratio of goat population to sheep population showed an increasing trend; 0.93 (CSA, 2012), 0.99 (CSA, 2015), 0.98 (CSA, 2017) and 1.05 (CSA, 2018); this might be an indication that

goats are becoming very important in Ethiopia. The increasing trend of goat population in Ethiopia is in line with prevailing situation in the world; Skapetas and Bampidis (2016) reported that the goat number in the world increased by 33.8% during the years 2000 – 2013. The increasing trend of goats' population could be associated with development of market for products from goats, changes of customers' attitude towards products from goats and the ability of these animals to adapt to a wide range of environmental conditions (Rodica et al., 2013). Increasing population pressure, land scarcity and diminishing production resources could be also promoters of goat in the tropics (Bett et al., 2009).

In developing countries, indigenous goats make valuable contribution, especially to livelihoods of the poor in the rural areas. They are important sources of meat, milk, manure, fibers & skins, and satisfy various cultural and religious functions (Tesfaye, 2004; Kosgey and Okeyo, 2007; Kanani, 2009; Aziz, 2010; Devendra, 2012). The importance of this valuable genetic resource is, however, underestimated and their contribution to the livelihood of the poor was inadequately understood (Kosgey et al., 2006; Kanani, 2009; Aziz, 2010).

With the help a project funded by SIDA (Swedish) and implemented by Biosciences for eastern and central Africa, International Livestock Research Institute (BecA-ILRI), a community based breeding programs (CBBP) on three indigenous goat breeds in Ethiopia were initiated in six villages in 2013 (Alubel, 2015; Tatek et al., 2016; Temesgen et al., 2019; Zergaw et al., 2016); currently, CBBP of small ruminants are being implemented in different parts of Ethiopia. The purpose of the project was improving goats' productivity in Ethiopia through efficient understanding of the indigenous goat genetic resources, genetic improvement, and enhanced individual and institutional capacity building. The goat breeds included Abergelle (AB), Central Highland (CH) and Woyto-Guji (WG). The CH inhabits the mid altitude or highland areas of the country where the annual rainfall is reasonably good while the AB and WG inhabited either arid or semi-arid agro-ecologies of the country. The CH, as the name indicates, is found in central highlands of the Ethiopia while AB and WG are found in the north and south ends, respectively, of the country.

Previous studies did not focus on assessments of does' productivity in terms of total weight of kids at three months per doe, survival of kids to three months of age and three month weight of kids per doe per year. Focuses were characterization of growth and reproduction traits from offspring's perspective. On the other hand, it was demonstrated that the indices focusing on weaning weights (three month weight in this case) per individual doe or ewe are superior for measuring the reproductive potential of meat breeds (Bosman et al., 1997). For instance, ewe productivity studies were available for Horro sheep (Duguma et al., 2002), Djallonke sheep (Gbangboche et al., 2006) and Nagerian goats (Bosman et al., 1997). However, such information is lacking for any of indigenous goat breeds in Ethiopia. Therefore, using monitoring data collected during the implementation phase of BecA-ILRI project, the objectives of the present study was to assess the productivity of Abergelle, Central Highland and Woyto-Guji does based on kids' total live weight and survival at three months of age. The total live weights of kids at three months were standardized by annum and post partum weight of does and factors affecting these parameters were investigated. Factors influencing the average daily milk yields of AB were also assessed.

Materials and methods

General description of data collection districts

Data used in the present study were collected in six villages located in five different districts in Ethiopia. The districts included *Tanqua Abergelle* of *Central Tigray* zone, *Ziqual* of the *Wag-Himra* zone, *Lay Armachiho* of *North Gonder* zone, *Meta-Robi* (now this district is divided into two: *Meta-Robi* and *Meta-Walkite*) of *West Shoa* zone and *Konso* Special district of *Segen zoria* zone in Ethiopia.

The AB are reared where the rainfall was uni-modal, short and erratic that extends not more than two months per year, usually from end of June to the end of August. Crop production usually fails due to low soil fertility and high moisture stress, almost every year (Alubel, 2015) signifying the importance of livestock in general and goats in particular.

About 50% of the land mass of *Lay Armachiho* district, one of the sites for the CH, lies between 1000 and 2300 meters above sea level (masl), while the remaining lies between 2000 and 3000 masl (Kahsay, 2013). In *Lay Armachiho* district the rain fall is uni-modal and usually starts from May to September. *Meta-Robi* district, in which the other village for CH was located, lies in a hilly land scope at elevations from 1,200 to 2,900 masl. Precipitation of this district was relatively low and mainly occurs during two seasons: the small rainfall, locally called *belg* rain, between March and April, and the big rain called *meher* rain between June and September.

The rainfall pattern of *Konso* district, where WG were reared, follows a bi-modal pattern there are two rainy seasons, i.e. *Belg* big rains with the period starting mid February and lasts to April and the small rain period *Meher* occurring around October and November (Cheung, 2008); the agro- ecological zones of *Konso* were 30% dry semi-arid and 70% arid. The temperature of the area is mostly experienced hot and warm that ranges between 12 to 33 degrees centigrade (Tesfaye, 2003).

Goat breeds and specific villages

AB had medium size (65-75 cm) height but stocky (28–34 kg); are mostly reddish brown in colour; males have magnificent spiral horns; the hair is short and smooth; all males have ruffs, and beards (94%) (FARM-Africa, 1996). The CH had a predominantly straight facial profile. All male goats have curved or straight horns which are oriented backwards. The coat colour of the breed is variable where the predominant colour being red brown with smooth hair. The mean height at the shoulders is 76.3 cm for adult bucks and 67.9 cm for does. Their mature body weight ranges from 30-43 kg (DAGRIS, 2007). According to Biruh (2013) the WG had their first kidding at about 22 months and 6.8 months of kidding interval. Netsanet et al. (2016) also reported a kidding interval of about 5.5 months for this breed.

Though maintained by the Agew and Tigray ethnic groups of northern Ethiopia, the AB were distributed along the *Tekeze* river in the provinces of southern *Tigray* (*Tembien and Inderta*), north *Wollo* (*Wag* and *Raya-Azabo*) and eastern *Gonder*; the climate is semi-arid to sub-humid with altitude above 1000 m; the production systems are mixed as well as agro-pastoral (FARM-Africa, 1996). WG Inhabits the regions of North and South *Omo* as well as parts of southern *Sidamo* and *Wolayta* in the southern Ethiopia; they are mainly kept by pastoralists ethnic groups (*Tsemay*, *Malie*, *Hamer*, *Benna*, *Dasenatch*, *Bumie* and *Guji*) and by a few agricultural societies (*Konso* ans *Gardula*); less distinct

types of this goat are also kept by the *Wolayta*, *Gofa* and Gamo people in North Omo; the climate is semi-arid and arid (FARM-Africa, 1996). According to the Awgichew and Abegaz (2008) the CH are mainly found in the central highlands, west of the Rift Valley, *Wollo*, *Gondar* and *Shoa*.

The villages for AB, CH and WG are located in *Tigray* and *Amhara*, *Amhara* and *Oromia* in and *SNNP's* (Southern Nations, Nationalities, and People's) region, respectively. Specific names for the villages were *Dingur* (*Tigray* region) and *Blaku* (*Amahara* region) for AB, *Waykaw* (*Amahara* region) and *Tatessa* (*Oromia* region) for CH and *Messale* and *Arkisha* (SNNP's) for WG. Study sites' identification was guided by the respective district agriculturalists. The location of these villages is detailed in Table 1.

Table 1. Latitude, longitude, altitude and rainfall of the study villages

Parameters	Dingur	Blaku	Waykaw	Tatessa	Massale	Arkisha
Latitude	$13^0 22'$	$12^0 81'$	$12^0 86'$	$9^0 54'$	$5^0 21'$	$5^0 26'$
Longitude	$38^0 98'$	$38^0 76'$	$37^0 35'$	$38^0 23'$	$37^0 26'$	$37^0 34'$
Altitude#	1731	1405	1192	2176	1383	1326
Rainfall (ml)*	710.65	546.95	1879.3	910.85	510.75	510.75

**=average rainfall of 2013 and 2014 (national Meteorology agency of Ethiopia) and meteorology stations for rainfall were Abi Adi, Sekota, Tikil Dingay, Ambo Agriculture, and Konso, from left to right, respectively; #=meters above sea level.*

Data collection

Ad hoc enumerators were hired to collect data of doe productivity traits. All does and doe kids of participating farmers were ear tagged with permanent plastic marker during mid July to August, 2013 at all the study sites; the data collection continued up to Mid April, 2015. Growth, mortality, and flock dynamics data were recorded. The growth traits being recorded were birth weight, three-month weight, six month weight, nine month weigh and yearling weights. While recording the mortality data, disease symptoms on a given animal and treatments provided with the dates and status (whether recovered or dead) were recorded. Data related to three-month weight and mortality to three months of age only were used in the present study. Other parameters including kidding interval (KI), litter size at birth (LSB) and productivity indices (index 1 and index 2) (detailed below) were derived from the already collected data.

Studied traits

In the present study five parameters, that could indicate the performance of doe, including total litter weight at three months (LWW), average daily milk yield (DMY), and three-month weigh doe productivity indices (index I and index II) and survival rates to three months were investigated.

Litter weight at three months: weights of kids born of a given parturition per doe and that reached three months after birth were considered. If multiple born kids survived up to three months, then the weight of all kids at this age points were summed up; otherwise, weight of single kid surviving to three months of age was recorded. In general, if there is no multiple births and no deaths, litter weight at three months is equal to weight at three months. If kid(s) born from a given doe could not survive until three months of age, then the value for litter weight at three months was zero; this was about

24% of the total kids in Abergelle goats, 22% of the total kids born in Central Highland goats and about 23% of the total kids born in villages for Woyto-Guji goat breed. Kids that did not survive until three months of age were ignored from the calculation of litter weight at three months.

Productivity indices: based on LWW and other literature estimates, productivity two productivity indices were investigated at individual does level (Bosman et al. 1997). This included productivity expressed as the body weight of a 90-day old kids produced per doe per year (index I) and per kg ppw per year (Index II). In this case, LWW were calculated as the sum of weights per parturition per doe at three months. The influences of breed, year of birth and parity of birth were investigated for the indices using General linear model procedure of SAS (2004).

$$y\ (Index\ I) = \frac{LW3M}{KI} \times 365 \quad \text{(Bosman et al., 1997; Peacock, 1987)} \qquad (1)$$

$$\text{Index II} = \frac{\text{Index I}}{\text{PPW}} \quad \text{(Gbangboche et al., 2006)} \qquad (2)$$

where y=live weight production per parturition in kg, standardized per annum, LW3M = Litter weight at three months after birth; KI=subsequent kidding interval.

Milk productivity of Abergelle does: average daily milk yield (DMY, ml) was collected from Abergelle goat breed for 12 weeks after kidding/parturition. A doe was milked once in a week starting a week from kidding/parturition date. Milking was twice per day: in the evening and in the morning. Kids were separately housed during night and farmers use traditional practice of tying teat and lubricating with dung to prevent kid suckling during day-time. When milk measurements were taken from a doe, her kid(s) was (were) assigned to another doe to ensure complete milking.

Survival rates to three months of age: the survival rates of kids born to a doe were analyzed using a logistic regression procedure in SAS (2004). From all kids born at a particular village during the study period that exited from the flock were recorded. Survival rate was calculated for kids that survived up to three months of age. In addition, kids that exited due to reasons other than deaths were considered as kids that survived. This was because had the reasons (other than deaths due to diseases) were avoided, the kids could have survived.

Fixed factors investigated

Fixed effects of villages, year, season, type, sex, and parity of kids' birth were investigated in the analysis of the studied traits where inclusion of the fixed effects was found to be important. Data were collected from two villages per breed and the effects of respective villages were investigated on the studied traits. Parity of a breeding female was known from owners during initial recording of flock at household level based on which subsequent parities were determined. Parameters with unknown parities were ignored from the evaluations of traits.

Seasons were categorized into 'dry' and 'wet' based on 2013 and 2014 rain fall data obtained from the national meteorology agency of Ethiopia. Accordingly, 'wet' months were July, August, and October in Dingur village; July, August and September in Blaku village; June, August, September, October and November in Waykaw village; April – October in Tatessa village; and January, March, June,

August, September, October and November in Massale and Arkisha villages. The rest months in the respective villages were categorized as 'dry' season.

Numbers of records were found to be unbalanced across year, type and parity of births; for instance, records from triplets, parity ≥ seven and in the year 2015 were found to be small at all the study sites. Due to these reasons, small records in 2015 were merged to data recorded in 2014; small triplet records (majorly for Central Highland goats) were merged to twin born ones and all parities greater or equal to parity six were considered as the sixth parity.

Data analysis and models fitted: general linear and logistic regression procedures of SAS (2004) were used for the analysis of the current data. Parameters including LWW, index I, index II, and DMY were analyzed using general linear model whereas survival rate to three months of age was analyzed using logistic regression model. The analysis of LWW, DMY, and survival of kids to three months (S3M) were made separately for the breeds whereas, in the analysis of index I and index II, data from the three breeds were combined and investigated. In the model fitted for the analysis of index I and index II parameters, fixed effects of breed, year of birth and parity of birth were fitted in the model. In the model for the analysis of LWW and S3M, fixed effects of villages, sex, year, type of birth, season of birth, and parity of birth were fitted; the model for the analysis of DMY contained fixed effects of villages, year of parturition, season of parturition and parity. In the analysis of S3M the logit model below was fitted:

$$\text{Logit}(p) = \ln\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1X_1 + \beta_2X_2 + \beta_3X_3 + \beta_4X_4 + \beta_5X_5 + \beta_6X_6 + \beta_7X_7$$

Where p=kid survival to three months; X_1, X_2, X_3, X_4, X_5, X_6, X_7 and X_8 were predictor variables of birth weight, post-partum weight, village, year, sex, birth type and parity, respectively.

Results

Total Litter weight three months (LWW)

The least squares means and standard errors of LWW for AB, CH and WG is given in Table 2. The overall least square mean of LWW (kg) for AB, CH and WG was 7.6, 15.3 and 10.2, respectively. Fixed effects of type and year of birth had significant effect ($p<0.001$) on LWW of all the three breeds. Multiple births resulted in significantly higher LWW compared to single births in all the cases. The LWW from multiple births were about a fold of LWW from single births (12.5 vs 7.8 for AB; 19.4 vs 11.6 for CH; 17.4 vs 9.2 for WG) (Table 2). On the other hand, the significant effect of year of birth on LWW was not constant for the three breeds; for instance, higher LWW were from births in 2013 for AB (10.6 kg in 2013 and 9.6 kg in 2014) and WG (13.6 kg in 2013 and 12.9 kg in 2014), whereas, in the case of CH, higher LWW was from kids born in 2014 (16.1 kg) compared to that of 2013 (14.8 kg). The effect of village of birth on LWW was significant in the case of WG only (14.9 kg in Arkisha and 11.6 kg in Massale). Likewise, season of birth significantly affected the LWW of AB only (10.6 kg in wet season and 9.7 kg in dry season). The LWW was not significantly affected by villages of birth in the cases of AB and CH. The effect of sex and parity of birth on LWW was not significant in all the three breeds. The effect of post partum weight on the LWW was found to be significant in all the three breeds.

Average daily milk yield (DMY)

Least squares means of ADM is given in Table 3 for Abergelle breed. The overall mean of the trait was 367.10±139.79 ml. Villages in which does kidded and milked had significant ($p<0.001$) effect on

DMY where does kidded and milked in *Dingur* had higher DMY (453 ml) compared to the other village (308 ml). Year and season of kidding did not significantly (p>0.05) affect DMY. On the other hand, parity of does significantly (p<0.05) affected DMY where DMY from the first parity was significantly smaller (354 ml) than DMY from the latter parties (378 ml to 390 ml).

Survival to three-month (S3M)

Survival rate to three months (S3M), likelihood ratio of the overall model and individual predictors for the three indigenous Ethiopian goat breeds is given in Table 4. The S3M was 76.30, 78.50% and 77.70%, for AB, CH and WG, respectively. The significant effect (P<0.001) of the likelihood ratio for the three breeds indicate that the fitted model was better than empty model. Intercepts were significant (p<0.001) in AB indicating that the logistic regression model could contain the constant whereas constant model can be constructed in the other two breeds. Village, parity, year and covariate PPW significantly (p<0.05) improved the fitted model for the analysis of S3M in AB. In the case of CH and WG, year of birth only significantly (p<0.05) improved the fitted model for the analysis of the S3M. Concerning the individual predictors including sex and type of birth did not significantly (p>0.05) improve the model for the analysis of S3M in all the three breeds. In addition, village, parity and constant PPW did not significantly (p>0.05) improve the fitted model for the analysis of S3M in CH and WG breeds.

Table 2. The effect of location, year, sex, season, birth type, and parity of birth and post partum weight on total litter weight at three months of age (LWW, kg) for Abergelle, Central highland and Woyto-Guji goat breeds in Ethiopia.

Parity	Abergelle		Central Highland		Woyto-Guji	
	N	LSMEAN±SE	N	LSMEAN±SE	N	LSMEAN±SE
Overall LWW	875	7.6±1.54	567	15.3±4.0	464	10.2±1.86
Village	Ns		Ns		***	
1	339	10.2±0.18	246	15.2±0.29	187	11.6±0.19
2	536	10.1±0.19	320	15.8±0.30	277	14.9±0.18
Year	***		**		***	
2013	536	10.6±0.19	158	14.8±0.36	146	13.6±.21
2014	339	9.6±0.19	408	16.1±0.22	318	12.9±0.17
Sex	Ns		Ns		Ns	
Male	441	10.2±0.18	326	15.7±0.26	264	13.4±0.17
Female	434	10.1±0.19	240	15.3±0.29	200	13.2±0.19
Season	***		Ns		Ns	
Dry	813	9.7±0.15	159	15.4±0.35	226	13.1±0.19
Wet	62	10.6±0.25	407	15.5±0.22	238	13.4±0.17
Birth type		***	***			***
Single	848	7.8±0.10	323	11.6±0.30	419	9.2±0.11
Multiple	27	12.5±0.31	243	19.4±0.30	45	17.4±0.29
Parity	Ns		Ns		Ns	
1	190	10.1±0.21	111	15.2±0.45a	111	13.3±0.23
2	110	10.2±0.23	109	15.6±0.41a	98	13.4±0.24
3	170	10.2±0.21	109	15.8±0.40a	84	13.3±0.24
4	167	10.3±0.21	92	15.7±0.45a	77	13.3±0.24
5	144	10.1±0.21	65	15.6±0.52a	54	12.9±0.28
6	94	9.9±0.23	80	15.1±0.48b	40	13.6±0.32

*N=number of observations (observations in LW3M were equal to observations in LSB in respective breeds and factors); ***=p<0.001; **=p<0.01; *=p<0.05; ns=p>0.05; z=least square means with different letters are significantly different; LSMEANS=least squares means; SE=standard error; ¥=1=Dingur, Waykaw and Massale for AB, CH and WG breeds, respectively and 2=Blaku, Tatessa and Arkisha for AB, CH and WG, respectively*.

Table 3. Least squares means of average daily milk yield (ml) in Abergelle goat breed

Factors		Abergelle	
		N	$\bar{X}$±SE
Overall		1150	367.1±139.78
Village		***	
	Dingur	519	453.4±7.17a
	Bilaku	631	308.1±8.22b
Year		S	
	2013	556	377.9±8.20
	2014	594	383.6±7.01
Season		Ns	
	Dry	999	374.4±4.61
	Wet	151	387.1±12.03
Parity		*	
	1	243	354.1±10.33b
	2	166	394.3±12.06a
	3	199	386.0±11.02a
	4	219	381.0±11.34a
	5	191	378.4±11.34a
	≥6	132	390.7±12.86a

Log odds of S3M and odds ratio of survival to death of S3M for factors having significant effect in at least one breed is given in Table 5. Log odds and odd ratios for S3M were 0.15 and 1.16, respectively for PPW in the case of AB. The log odds and odds ratios for the trait in AB were -1.60 & 0.20 and 1.86 & 6.43 for *Dingur* compared to *Blaku* and for 2013 compared to 2014, respectively. The range of log odds and odds ratio for parity 1 to 5 compared to parity 6 was 0.38 – 0.96 and 1.47 to 2.60, respectively for AB. Log odds and odds ratio of S3M for the year 2013 compared to 2014 were -0.20 & 0.82 and 0.81 &2.24 for CH and WG, in respective order.

Table 4. Survival rate at three months of age, significance of likelihood ratio and individual predictors in the logit model for three indigenous Ethiopian goat breeds under farmers' production practices

Breed	n	S3M	LLR	INT	Ppw	vil	Year	Sex	btype	Parity
AB	1152	0.763	***	-3.82***	***	***	***	Ns	Ns	*
CH	991	0.785	***	0.47ns	Ns	Ns	***	Ns	Ns	Ns
WG	649	0.777	*	1.38ns	Ns	Ns	**	Ns	Ns	Ns

*S3M= survival rate to three months of age; LLR= log likelihood ratio; INT= intercept; bwt= birth weight; ppw= post-partum weight; vil= village; btype =birth type; n= number of observations; *** = p<0.001; **=p<0.01; *p<0.05; ns=p>0.05; AB= Abergelle; CH= Central highland; WG=Woyto-Guji.*

Table 5. Changes in Log odds of survival and odds ratios of survival to deaths at three months of age in three Ethiopia indigenous goat breeds under farmers production practices

Predictors		Abergelle		Central Highland		Woyto-Guji	
		Log odds	Odds ratio	Log odds	Odds ratio	Log odds	Odds ratio
Postpartum weight		0.15±0.03	1.16	-0.003±0.02	0.99	0.01±0.03	1.01
Village (1)		-1.60±0.118	0.20	0.06±0.24	1.06	0.006±0.26	1.01
Year (2013)		1.86±0.19	6.43	-0.20±0.19	0.82	0.81±0.25	2.24
	1	0.96±0.29	2.62	-0.002±0.36	0.99	-0.27±0.40	0.76
	2	0.53±0.31	1.69	0.05±0.33	1.05	-0.61±0.40	0.54
Parity	3	0.79±0.31	2.20	0.12±0.32	1.13	0.05±0.40	0.96
	4	0.44±0.29	1.56	-0.28±0.30	0.76	-0.03±0.34	0.97
	5	0.38±0.30	1.47	-0.04±0.33	0.97	0.55±0.47	1.73

Productivity indices

Three-month weight productivity per year per doe (index I) and per year per doe ppw (index II) is given in Table 6. Overall means of index I and index II were 14.7 kg and 0.50, respectively. The CH breed had significantly ($p<0.001$) higher index I (22.3 kg) and index II (0.50) followed by the WG breed (13.1 kg of index I and 0.52 of index II). Index I was significantly ($p<0.001$) higher for births in 2014 (15.9 kg) than births in 2013 (13.2 kg); similarly, index II was significantly ($p<0.05$) smaller for births in 2013 (0.49) than births in 2014 (0.54). Earlier parities (parity 1 and 2) were characterized by significantly ($p<0.001$) lower index I (12.2 kg to 13.0 kg) than latter parities (15.2 kg to 16.3 kg). However, the effect of parity of birth was not significant ($p>0.05$) on index II.

Table 6. The influence of year and parity of previous parturition and breed on least squares means of three months weight productivity of does (index I and index II)

Parameters	Index I		Index II	
	N	LS means ± S.E	N	LS means ± S.E
Overall means	731	14.7±7.32	720	0.50±0.22
Breed	***		***	
Abergelle	345	8.4±0.41c	337	0.33±0.01c
Central Highland	295	22.3±0.44a	292	0.69±0.01a
Woyto-Guji	91	13.1±0.77b	91	0.52±0.02b
Year	***		*	
2013	333	13.2±0.47b	327	0.49±0.01a
2014	398	15.9±0.42a	393	0.54±0.01b
Parity	***		Ns	
1	78	12.2±0.85b	78	0.51±0.02
2	106	13.0±0.72b	104	0.49±0.02
3	159	15.6±0.59a	158	0.54±0.01
4	168	15.3±0.58a	164	0.51±0.01
5	120	16.3±0.69a	117	0.52±0.02
≥6	100	15.2±0.77a	99	0.49±0.02

Discussion

Twin born kids had significantly ($p<0.05$) heavier LWW in all the three breeds. Kids born and reared in *Dingur*, *Tatessa* and *Arkisha* villages for Abergelle, Central highland and Woyto-Guji breeds, respectively had heavier LWW. Abergelle and Woyto-Guji kids born in 2013 and Central highland kids born in 2014 had significantly ($p<0.05$) heavier LWW than kids born in the other year. The overall mean of total weight of lambs for Horro sheep was about 24.3 kg (Duguma et al., 2002) considerable higher than even the LWW for CH goats in the present study. This could be due to the general fact that the three-month weight of sheep is greater than the three month weight of goats; in addition the litter size of Horro sheep at three months of age after parturition could be higher than the litter size of goat breeds studied presently.

Breed and year of birth had significant effect on index I and Index II. Index I of Nigerian goat reported by Bosman et al. (1997) was smaller (8.3 kg – 10.2 kg) than any of the values of index I in the present study (8.4 – 22.3 kg) regardless of their shorter parturition intervals (260 – 279 days) than that of AB and WG of present study (305 – 348 days). This was probably because the weaning weights composing the indices calculation in Bosman et al. (1997) (4.7 – 5.8 kg) was smaller than that used in the present study (7.39 – 10.94 kg). As indicated in equation 1, index I was calculated as the ratio of three-month weight (3mw) to KI. This means higher 3mw and smaller KI leads to higher index I. In the reports of Gbangboche et al. (2006), the weight used as 3mw were about 10.62 kg (higher than almost all of the values in the present work) and lambing intervals of 0.665 years, shorter than the kidding interval values reported in this work, hence, considerably higher values of index I than ours in Gbangboche et al. (2006) was reported. In agreement with our report, parity and year of birth had significant ($p<0.001$) on the index I in Gbangboche et al. (2006) where higher values were associated with older parities. The overall mean of index II for Djallonke sheep was 0.56 kg (Gbangboche et al., 2006), higher than overall mean value (0.50) in our work. In general, higher index I and index II were associated with heavier 3mw and ppw and shorter KI. These indices showed significant difference for the fixed effect of breed where the highest values were for CH followed by WG. High values from CH breed could be associated with the desired values (higher 3mw and shorter KI were the desired values for instance) of the parameters used to calculate the indices.

The DMY in the present study is lower than values reported by Alsheikh (2013) and comparable with reports of Mahal et al (2013). As in the case of the present study parity had significant effect on milk production of Black Bengal goat (Goetsch et al., 2011). Milk production generally reaches peak in mid parities and decrease thereafter (Goetsch et al., 2011). Similar to the present study, season of kidding did not significantly affect milk yield of goat (Mahal et al., 2013; Bushara et al., 2013). However, amount of daily milk yield was high in the rainy season probably due to the effect of quantity and quality of feedstuff provided by pasture in the rainy season, or due to presumably benefitted nutritionally from leaf development by some browse species (Bushara et al., 2013).

The survival rate in the present study was in agreement with reports of Akpa et al. (2009), lower than reports of Hailu et al (2006). The survival rates reported in the present study were higher than the survival rate of kids reported by Husain et al. (1995). Regarding effects of fixed factors variable reports are available; in the studies of Hailu et al. (2006) fixed effects of year, parity, breed type, sex, season of birth, litter size and birth weight significantly ($p<0.05$) influenced survival rate of Borana and Arsi-Bale kids; in reports of Husian et al. (1995), fixed effects of birth type and sex were not

significant whereas year of birth was significantly influencing survival to weaning of Black Bengal kids; however, in the present study year of birth for all the studied breeds and location and parity of birth for Abergelle breed had significant ($p<0.05$) influence on survival of kids to three months of age. The better survival rates based on log odds and odds ratio values of Abergelle and Woyto-Guji kids in 2013; the better survival rate for Central highland breed in 2014 was reflected by favourable factors for kids in that year.

Conclusion

The CH does produced about double LWW of AB does and about 150% LWW of WG does. They also had the highest productivity indices (index I and index II) followed by WG does. The kids from the CH had the highest survival rates. On the other hand, AB does best suited for dual services (producing kids for meat and production of milk). Taking the present findings into considerations, investment priorities in Ethiopia focusing on goat meat production could target the CH goats. However, as any of the breeds could not thrive out of their niche, replacing one breed by another should not be an option. Rather, the productivity of the indigenous goat breeds should be optimized in their respective localities by minimizing possible factors hampering their productivity. As improving productivity through efficient understanding of the indigenous goat genetic resources is very crucial, knowledge of factors significantly affecting does' productivity is very helpful while implementing the CBBPs of goats.

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Open Nucleus Breeding Strategy for Fogera Cattle Breed in Ethiopia: Achievements and Future Directions

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Abstract

Indigenous cattle breeds constitute an important reservoir of genetic material which developing nations have failed to give adequate recognition. Fogera cattle are one of the promising indigenous milk type local breed of Ethiopia that is widely adapted around Lake Tana of Amhara region. The objective of this paper was to evaluate the performance of nucleus herd at Andassa livestock research center and to evaluate and summarize the result of first term community based breed productivity improvement (CBBPI) done under open nucleus herd breeding scheme. The work was done in two purposively selected districts (Gondar Zuria and Fogera). The collected data was analysed by GLM procedure of SAS (2002). The overall milk yield of the nucleus herd was 2.26±0.794 litter. From the total herd, best 10% and 25% of them give a respective milk yield of 3.31 and 2.87 litter; and some elite cows give an average of 5.45±0.73 litter with a maximum yield of eight litters per day. The overall birth and weaning weight of nucleus herd calves was 21.30±0.06 and 103.66±0.59kg, respectively. For CBBPI program, 17 pure Fogera bulls were distributed to the two districts. The birth and weaning weight of the village calves was 23.77±0.21 and 85.89±1.07, respectively. Through the program, above 1000 households were benefiting through the breeding program, health, forage development and grazing land management. To share the achievements of the results in CBBPI program, popularization through filed days and innovation platforms; and documentation of each step via publications, videos and pictures was done. From the result, it can be concluded that the strategy implemented for the breed conservation "open nucleus breeding scheme" had shown better results and improvement was recorded. To widen the result and improve the livelihood of the farmer, scaling up of the activity should better be planned with integrated approach of different stakeholders and strong linkage and follow up of the community should be done.

Key words: Community based, Fogera cattle, Nucleus herd, Selection response

Introduction

Indigenous cattle breeds constitute an important reservoir of genetic material which developing nations have failed to give adequate recognition. Changes in economic situation, the changing consumer preference and therefore the need for change in production methods to comply with these are the major forces dictating the future of indigenous cattle breeding (Assan, 2012). Today, a large number of indigenous breeds or varieties in the developing world are at risk of becoming extinct; likewise, at the moment breeds like Sheko cattle, are highly threatened as a result of interbreeding; others like Fogera, Begayit, Ogaden and Borena cattle breeds of Ethiopia are also facing various degrees of threat (IBC, 2004). These breeds are decreasing and deteriorating in terms of both population size and genetic quality due to paradigm shift in the existing farming system and production system, and farm size dynamics of the native habitat and in turn the subsequent genetic dilution (IBC, 2012; Adebabay et al., 2013), those directs a need for genetic conservation.

Fogera cattle are one of the promising milk type local breed of Ethiopia that is widely adapted around Lake Tana of Amhara region. The breed in known for its relatively higher milk yield and traction power, better resistance to internal parasites infestation, and sound adaptability to water logged Fogera plain attributed to its long legs (Assemu Tesfa, 2015). On top this, milk production and draught power merits of the breed are farmer's preferable traits (Zewdu, 2004; Addisu *et al*., 2007). Currently the breed is used as Dam line for milk yield improvement (with Holsten Frisian bull) and Sire line to improve the considerable milk yield and better growth rate for rural areas and water logged areas (Assemu Tesfa et al., 2017).

Andassa livestock research center had exerted an improvement and conservation effort both on-station and on-farm level to safeguard the breed from extinction and decrement in its productivity. The center keep the breed for above 40 years as a conservation effort and improvement activity both in-situ and ex-situ for the past 20 years through open nucleus breeding scheme. The on farm conservation and improvement effort was done both by own animal selection for 10 years and by back crossing with pure Fogera bull since 2013. The final objective of the breeding scheme was restocking the declining village Fogera cattle population and improving the livelihood of the farmer. Therefore, the objective of this paper was:

- To evaluate the performance of nucleus herd at Andassa livestock research center
- To evaluate and summarize the result of first term community based breed productivity improvement done under nucleus herd breeding scheme of Fogera cattle.

Materials and Methods

Description of working sites

Andassa Livestock Research Center (ALRC): is found at 587 km Northwest of Addis Ababa, and 22 km South of Bahir Dar city (capital of Amhara region), on the way to Blue Nile fall. The total area of the center is about 360 hectares out of which 310 are covered by pasture land and the rest 50 hectares is covered with bushes and different constructions. The center was established to conserve Fogera breed both in-situ and ex-situ approach. The center had above 600 nucleus herd animals for conservation and improvement strategy. Community based breed improvement program was implementing at two districts; those are known to have true-to-type fogera cattle breed. These are:

Gondar Zuria district: The altitude of the district is 1982 masl and the average annual rainfall range between 950 to1035mm. The annual temperature ranges from 27^{o}c to 33^{o}c. The total area coverage of the district is 114,983ha. The cattle population was estimated to be 212,164 (AGADO, 2014).

Fogera district: Altitude ranges from 1774 to 2410 masl, and receives mean annual rainfall of 1216.3 mm. It has an estimated cattle population of 182729. The land, about 44.2% is arable and 20% is irrigated, 22.9% for pasture, 1.8% forest or shrub land, 3.7% is water, and the remaining 7.4% is considered degraded land.

Description of Fogera breed

Fogera breed is characterized and well known by its pied coat of black-and-white or black-and-grey; short, stumpy, pointed horns; hump ranges from thoracic to cervico-thoracic; dewlap is folded and moderate to large in size; docile temperament; used for draught, milk and meat (Rege and Tawah, 1999;

DAGRIS 2007). It is highly tolerant or resistant to heat stress and solar radiation which could be due to its light color and short hair. Additionally, the breed is known for its tolerance to high altitudes, parasite and disease infestation, fly burden, wet soils or swampy areas, low quality of feed and other unfavorable environmental conditions (Alberro and Haile-Mariam, 1982).

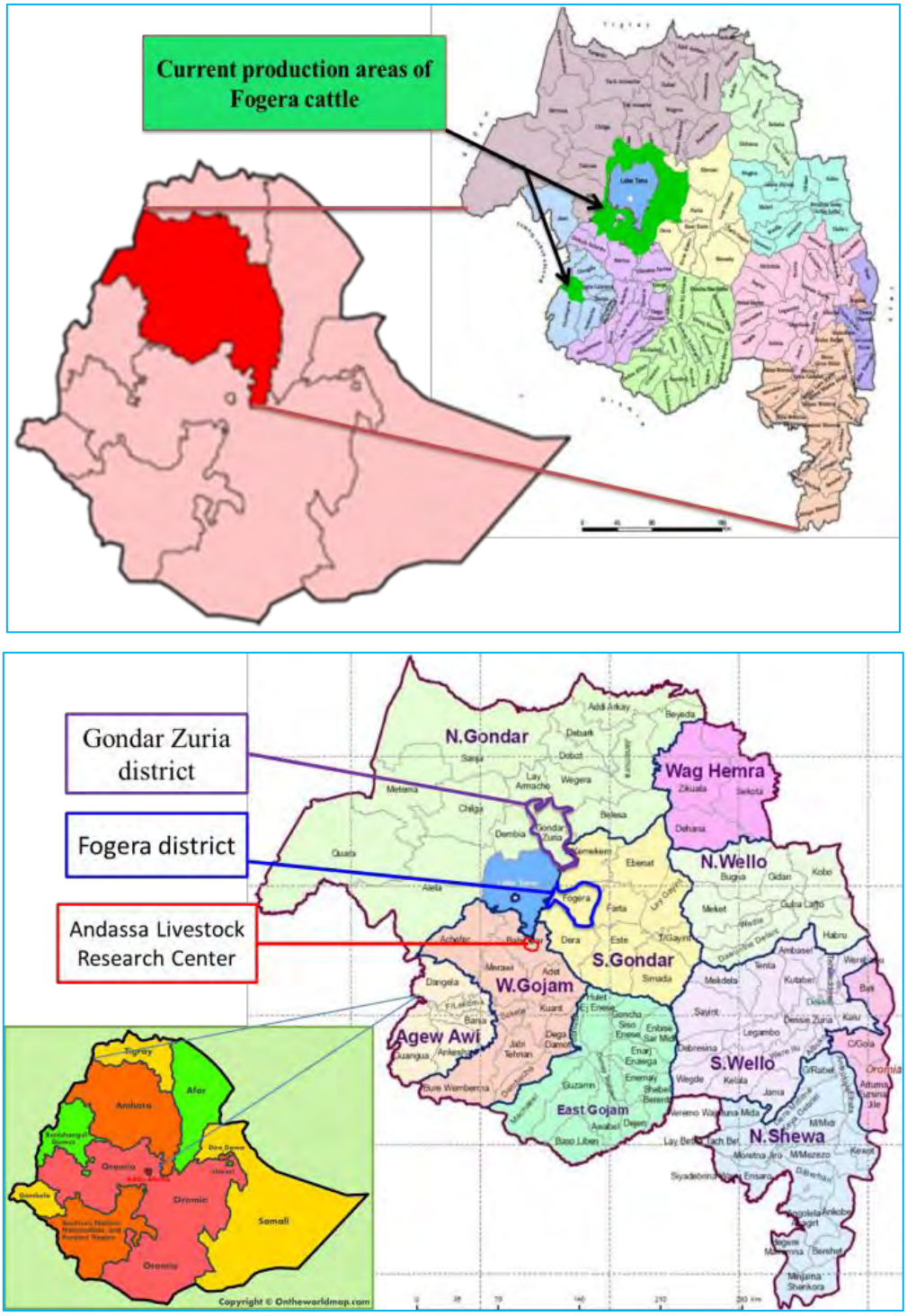

Figure 1. Distribution of Fogera cattle (left) and working sites (right)

Breeding strategy

The center follows open nucleus breeding scheme (figure 2), on which improved bulls from the nucleus had transported to community (village) herd and selected heifers to the nucleus herd. In the nucleus herd, animals are grouped based on their milk yield and pedigree. A single herd had 40 to 50 cows with one bull and mating is natural. Calves had free access to suckle their dams for the first four days to ensure that they consume enough colostrum; they were then separated from their dams and allowed to partially suckle (two teats) at milking times until weaning.

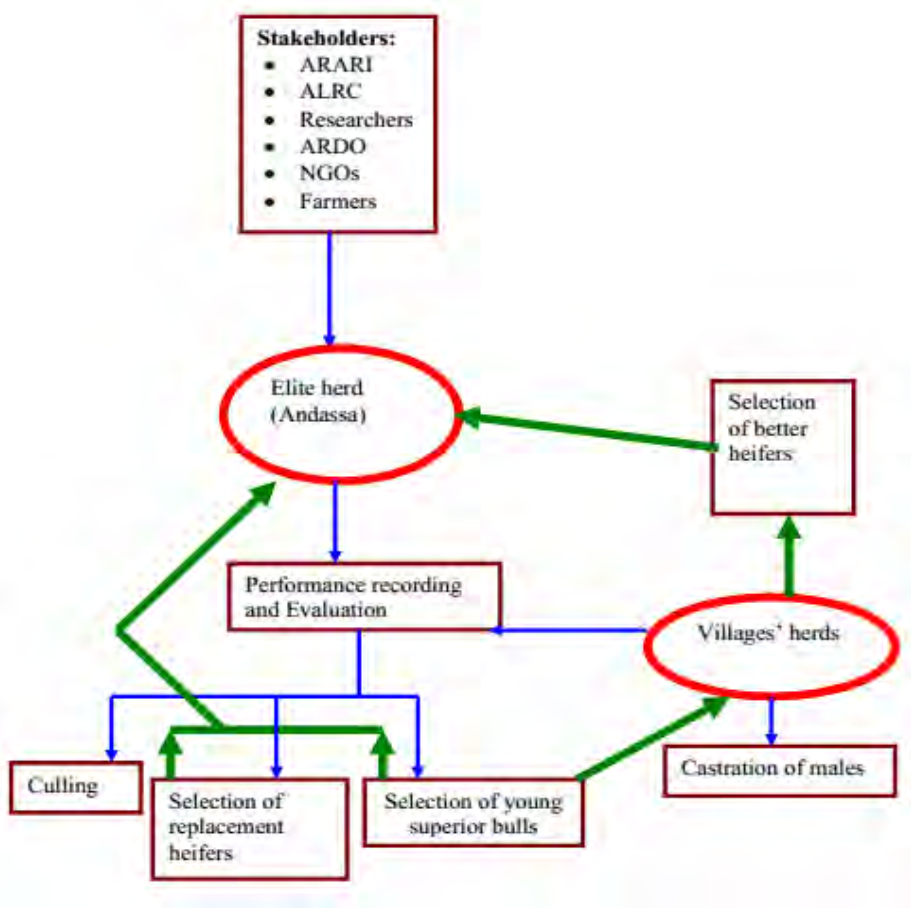

Figure 2. Open nucleus breeding scheme

Community Based Breed Productivity Improvement (CBBPI)

As a part of open nucleus breeding strategy, community based breed productivity improvement is the implementation program of the strategy at the village herd. For the implementation, through participation of researchers and experts, two districts based on presence of true to type Fogera cattle (50%), Accessibility and presence of knowledgeable farmers (25%) and others like willingness of farmers, communal grazing land and enough land for feed development (25%) was selected. After

selection, community discussion was done on points like the importance and productivity of the breed, its value for them and the need of the conservation and improvement strategy. After the consensus built with the community, farmers were selected to hold the breeding bull and serve the community. Those farmers were selected based on wealth status, cattle management ability, and presence of better educational background. And the bull was given based on written contractual agreement for four years' service and after to make him own property.

Data analysis

The collected data was analysed by GLM procedure of SAS (2002) software. Milk yield performance, and pre-weaning growth was considered as dependent variables; breeding period and breeding group for nucleus data and sex, season, district and year for community based data was used as independent factors, respectively. Dependents of growth and reproductive performances

Result and Discussion

Part I: Nucleus herd achievements

Milk yield performance

The result of the data (Table 1) indicated that through the advancement of year, milk yield was improved mainly since from 2008. The lowered milk yield during the period of 2005-2007 was due to the recorded inbreeding in the herd. The gradual increment was due to culling and restocking procedure done by the center. From the total herd, 10% and 25% of them had milked a respective average milk yield of 3.31litter and 2.87 litter (Table 2); this result had an increment compared with the average milk yield of 3.04 and 2.62 litter (Addisu *et al*., 2010) for best 10% and 25% of the herd. As table 3 presents, the group herd had variations in milk yield performance as they are group based on their milk yield performance. Among the entire herd, some elite cows give an average of 5.45±0.73 with a maximum yield of eight litters; and this indicated that the selection response of the breed is higher.

Table 1. Least square means of daily milk yield in different periods

Parameter	N	Daily Milk Yield	Standard Error
Period 1 (2002-2004)	13958	2.30	0.009
Period 2 (2005-2007)	8504	1.92	0.008
Period 3 (2008-2010)	31657	2.13	0.008
Period 4 (2011-2013)	10754	2.39	0.01
Period 5 (2014-2017)	10114	2.43	0.071

Table 2: Average daily milk yield performance and Predicted milk yield of Fogera cattle

Parameters	Daily milk yield	Predicted Milk yield at100days	Predicted Milk yield at 200days	Predicted Milk yield at 305days
Overall mean	2.03	242.82	430.18	578.26
Best 10% mean	3.31	380.44	669.89	883.64
Best 25% mean	2.87	326.75	585.32	772.83
Maximum	4.55	493.50	858.00	1194.00
Minimum	0.56	78.00	175.00	274.00

Table 3. Milk yield performance of the grouped herd

Group	Herd type	Milk yield (litter)			
		Mean	SD	Min	Max
	Overall	**2.26**	**0.794**	**0.4**	**7.2**
1	Fogera G 1	2.24	0.731	0.7	5.5
2	Fogera G II	2.52	0.863	0.6	6.1
3	Fogera G III	2.05	0.814	0.4	7.2
4	Fogera G IV	2.18	0.707	0.6	4.5

Birth and weaning weight performance

The overall birth and weaning weight of nucleus herd calves was 21.30±0.06 and 103.66±0.59kg, respectively. The result was comparable with the report of Giday (2003) and Assemu Tesfa (2015). As indicated in table 4, the result had shown an improvement from year to year which is the response for selection of the breed.

Table 4. Least square means of birth and weaning weights by sex and year group

Parameters	N	Birth weight (kg)	N	240 day weight
Overall	**1975**	**21.30±0.06**	**1426**	**103.66±0.59**
Sex		*		**
Female	1131	20.92±0.09	805	101.91±0.93
Male	1075	21.86±0.09	766	108.50±0.95
Year		***		**
1997-2000	438	$21.67±0.14^{b}$	408	$107.87±1.11^{b}$
2001-2004	385	$21.80±0.14^{b}$	340	$102.76±1.21^{c}$
2005-2008	408	$21.07±0.14^{c}$	271	$94.73±1.36^{d}$
2009-2012	421	$19.53±0.14^{d}$	337	$104.53±1.22^{bc}$
2013-2015	323	$22.88±0.16^{ab}$	70	$116.14±2.67^{a}$
2016-2018	231	$23.83±0.59^{a}$	145	$101.45±1.71^{c}$

Part II: Community based breed productivity improvement (CBBPI)

The approach, CBBPI, enables to establish a linkage between ex-situ and in-situ conservation strategies (nucleus herd and the village herd), allows to maximizes the retention and continued evolution of the genetic qualities of farmers' varieties, aims to avoid the loss of variation during rejuvenation and maintenance in formal gene banks and finally its benefit was mainly due to because of community is responsible for the decisions on definition, priority-setting and the implementation of all aspects of its conservation and sustainable use.

Birth and weaning weight achievements

Since 2012, the center had work on village breed improvement from the entire herd of the community. Bus after 2013, the implementation of open nucleus breeding scheme was done and selected bulls from nucleus herd were distributed to the selected villages. Since then, 17 pure Fogera bulls were distributed in two terms for both working districts. In first term (four year period), the mating bulls were transferred to the keeping farmers based on the agreement. The birth and weaning weight of the village herds born from CBBPI was 23.77±0.21 and 85.89±1.07, respectively (Table 5). The average weaning age was lowered from one year to 8 months. Based on the monitoring data the average birth and weaning weight of calves born by local bull of the area was 20.21 and 85.14kg (at one year age), respectively. From this it can be appreciated that, the through the open nucleus breeding program, there was an improvement in productivity beyond the conservation effort.

As a strategy, community based breed productivity improvement, includes different aspects of activity besides the breeding program. These activities, done in relation of the breeding program was strategic deworming and vaccination through flock monitoring strategy, grazing land improvement and introduction of new water logged tolerant grass specious and fattening technology demonstration; those all allow to improve the production improvement and the livelihood of the farmers. Above 1000 households are benefiting in the strategy through the CBBPI. Through the first term of the breeding program, above 38 castrated bulls fattened; above 24,000 cattle covered by health treatment and Elephant grass, four water logging tolerant grass cultivars were introduced to alleviate feed problem of the area.

Case report: second generation of the strategy had reported and age at first mating was reported to be 25 month and 36 months as age at first calving. This result compared with the farmers calve is improved by an average of one year, which indicates the positive response of selection done on the breeding strategy. As traction power is the selective trait of the farmers, calves born through the CBBPI starts plowing in their 31 months while the farmers local bull do the same work at its 41 months age.

Popularization, documentation and Farmers perception on CBBPI

To popularize the activity and increase the participant farmers and districts, field day with different stakeholders of about 228 participants (BoA, Universities, IBC, zone to kebele offices, farmers and NGO) was held mainly to indicate the efforts exerted on the breed improvement and conservation; share tasks for "hands together" for the use of the resource; popularize the activity and develop scaling up modality to other target areas.

Table 5. Least squares means (+SE) of birth and weaning weights of Fogera cattle by location, year, sex and season

Parameters	N	Birth weight	N	Weaning weight
Overall	**567**	**23.77±0.21**	**293**	**85.89±1.07**
District		*		**
Fogera	372	24.01±0.26	190	86.44±1.29
Gondar zuria	195	23.31±0.33	103	84.54±1.92
Year		**		*
2015	207	23.08±0.33ab	108	86.16±1.53b
2016	158	24.17±0.34a	87	85.56±1.48b
2017	107	24.52±0.36a	98	87.49±3.05a
2018	95	21.75±0.62c	-	-
Sex		*		**NS**
Male	277	24.15±0.30	137	86.60±1.84
Female	290	23.45±0.27	156	85.30±1.24
Season		**NS**		*
Dry	388	23.27±0.35	209	84.27±2.35
Wet	179	23.75±0.56	84	87.51±1.05

As CBBPI activities are long lasting, documentations had been done in every and each journey of the activity, which allow to see the drawbacks, identified challenges, take corrective measures, and further developing guidelines to other users and share the experiences to other areas. In this regard, CBBPI activity on Fogera cattle had documented in different forms viz. via videos, photos, publications (journals and proceedings) and other written documents.

Participant farmers, getting a calve from the improvement effort, had *"acknowledged* Almighty *GOD for having the cattle phenotype of their elder fathers".* Some farmers were reluctant to show their calves to us due to *their fear as we take off the calves to the center*. All farmers are well satisfied by the service delivered in relation to the conservation and improvement activity to improve the production environment. Non-participating farmers of neighboring gotes, PA's and districts requests the research center to expand the conservation and improvement work with their vicinity.

Major Challenges of community-based breed productivity improvement of Fogera Cattle

1) Over flooding of Lake Tana - the lake, always over flood to the grazing land and make stagnant water for several months that lead disease outbreak and damage on the grazing land.
2) Invasive weeds - in both sites, the grazing land had covered by unpalatable weeds like Amekela (*Asracantha longifolia*) and water body invasive weeds (water hyacinth) share the land and aggravate disease (mainly water hyacinth).
3) Shift of production system - the dominant livestock-crop mixed production system of the areas was currently dominated by crop production and diversification that change the grazing lands to crop production.
4) Genetic erosion - intact male Fogera bulls are currently get better price to the neighbouring countries market. This, with absence of marketing policy for border live animals, challenges both the conservation and improvement strategy.

Conclusion

- In the current study, the result across year and period had shown an inconsistence result; which might be attributed to the presence of variable management practices adopted by the center.
- There is an improvement in the production performance of the breed that indicated the potential for selection is higher in the breed.
- The community conservation and improvement strategy was the main entry point to assure the conservation effort.
- Cumulative effort viz. breeding, feed, health and extension play a great role to sustain the conservation and improvement effort.

Recommendations

With the above conclusions, the following recommendations are made to further strengthen the conservation and improvement effort of the Fogera breed.

- Strong linkage and follow up of the community should be done.
- The production environment of the conservation and improvement sites should be improved to improve the livelihood of the farmers through optional income generating activities
- Developing a reproductive technology and biotechnology unit to safe guard the breed from extinction by natural and/or manmade hazards had better be planed and implemented.
- Scaling up of the activity should better be planned with integrated approach of different stakeholders

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