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Grain Yield Potential

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Highlights

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Discovering Thoughts, Inventing Future

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Genetic Gain in Grain Yield Potential and Associated Traits of Tef [*Eragrostistef (Zucc.)*Trotter] in Ethiopia

By Fano Dargo, Firew Mekbib & Kebebew Assefa
Jigjiga Uinversity

Abstract- Evaluation of varieties from different years in a common environment is the most direct of the several methods that have been used to estimate breeding progress. 33 tef varieties released in Ethiopia since 1970 till now were evaluated at DebreZeit and Melkassa Agricultural Research Centers to estimate the amount of genetic gain made over time in grain yield potential and associated characters. The varieties were laid out in a randomized complete block design with three replications in 2012 cropping season. Analysis of variance revealed significant differences among varieties for all traits except hundred seed weight on both locations.Grain yield was increased from 3848.68 kg ha^{-1} to 4934.4kg ha^{-1} over the past 42 years. The average annual rate of increase per year for the period of 1970-2012 was estimated from the linear regression of mean grain yield on year of variety release was 21.53 kg ha^{-1} with a relative genetic gain of 0.56% $year^{-1}$ which was highly significantly different from zero.

Keywords: grain yield, harvest index, biomass yield, plant height, phenologic traits, yield attribute, productivity traits.

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Fano Dargo [α], Firew Mekbib [σ] & Kebebew Assefa [ρ]

Abstract- Evaluation of varieties from different years in a common environment is the most direct of the several methods that have been used to estimate breeding progress. 33 tef varieties released in Ethiopia since 1970 till now were evaluated at DebreZeit and Melkassa Agricultural Research Centers to estimate the amount of genetic gain made over time in grain yield potential and associated characters. The varieties were laid out in a randomized complete block design with three replications in 2012 cropping season. Analysis of variance revealed significant differences among varieties for all traits except hundred seed weight on both locations.Grain yield was increased from 3848.68 kg ha^{-1} to 4934.4kg ha^{-1} over the past 42 years. The average annual rate of increase per year for the period of 1970-2012 was estimated from the linear regression of mean grain yield on year of variety release was 21.53 kg ha^{-1} with a relative genetic gain of 0.56% year^{-1} which was highly significantly different from zero. Grain yield potential of tef has not attained plateau in Ethiopia. Thus, development of higher yielding varieties of tef should continue to increase tef grain yields if past trends pretend the future. Biomass yield also showed highly significantly increase with annual genetic gain of 73.74 kg ha^{-1} year^{-1}. Similarly grain yield per day and biomass production rate at both locations showed highly significantly different from zero except biomass production rate at Melkassa which was significantly increased with year of release. On the contrary, days to panicle emergency, days to maturity and lodging index at both locations showed non-significant decreasing trend over years. Linear regression also indicated that significant improvements in yield panicle^{-1} and panicle weight across location and at both location respectively. No marked changes were observed in days to seedling emergency, harvest index, plant height, panicle length and hundred seed weight during the study period which implied that tef improvement effort has failed to bring a substantial progress on these traits. Grain yield was significantly and positively associated with biomass yield, yield panicle^{-1}, grain yield day^{-1} and biomass production rate, where as there was no significant correlation between grain yield and phenologic traits, harvest index, plant height, hundred seed weight, panicle weight, lodging index and panicle length. Stepwise regression analysis revealed that most of the variation in grain yield of tef was caused by biomass yield and harvest index. In order to see the impact of the achievement in the genetic progress of tef research, it is imperative to undertake large scale popularization of the released varieties.

Keywords: grain yield, harvest index, biomass yield, plant height, phenologic traits, yield attribute, productivity traits.

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I. Introduction

Tef[*Eragrostistef (Zucc.)* Trotter] is an ancient crop in Ethiopia, and the country is considered to be center of both origin and diversity for the species (Vavilov, 1951). Its grain is gluten free, and is a good flour source for segments of the population suffering from gluten intolerance or Celica's disease (Spaenij-Dekking*et al*., 2005). It has gained momentum as a forage crop and several new, improved types have been developed and commercialized (Miller, 1995).

Tef belongs to the grass family Poaceae. It is a C4; self pollinatedchasmogamous annual cereal (Seyfu, 1993). It is an allotetraploidcereal crop with a chromosome number of 2n = 4X = 40 (Tavasoli, 1986). Tef is indigenous to Ethiopia and has an amazing wealth of diversity (Seyfu, 1991). In Ethiopia, tef is grown on more than 2.7 million hectares, (CSA, 2012).

Ecologically, tef can be grown in a wide range of environments, and is presently cultivated under diverse agro-climatic conditions. It can be grown from sea level up to 2800 m.a.s.l. (Seyfu, 1993).The ability of tef to perform well on both waterlogged vertisoils in the highlands as well as in low moisture stress areas in the semi-arid regions throughout the country is one of the reasons for which tef is preferred over other grain crops such as maize or barley (Hailu, 2001). In addition, tef generally suffers less from biotic stresses compared to most other cereal crops grown in Ethiopia and it contains high levels of proteins and mineral (Seyfu, 1993). Despite the aforementioned importance and coverage of large area, its productivity is very low (1.28 t ha^{-1}) (CSA, 2012). Some of the factors contributing to low yield of tef are lack of high yielding cultivars, lodging, weed, water logging, low moisture and low soil fertility conditions (Fufa, 1998).

Documentation of the contribution of plant breeding to a given crop yield improvement and evaluation of the past gains are useful for identifying areas with potential for planning a future breeding program (Waddington *et al.,* 1987). Evans (1993)

advocated that an understanding of changes produced by crop breeding on grain yield and its determinants was important to evaluate the efficiency of past improvement work to facilitate further progress.

Genotype, environment and management interact to determine the yield of a crop. However, no method of estimating long term improvement progress can completely separate genetic effects per se and their interaction effect. Nevertheless, evaluation of popular cultivars from different years in common environments is the most comprehensive and direct method that has been used to estimate progress in yield improvement. Progress made in grain yield potential and associated traits produced by genetic improvement have been documented in different crops in different countries (Perry and D'Antuono, 1989).

The national and regional agricultural research system has been striving to improve tef production in Ethiopia since the late 1970's, and 33 varieties of tef have been released so far for commercial production from 1970 until 2012. Yifru and Hailu (2005) reported study conducted on genetic gain of tef in 1997. Their study was involved one farmer variety and 10 improved varieties released over the periods 1970-1995. However, the progress made in breeding of these varieties after 1995 and two location trials were not assessed. Therefore, the present study were undertaken to overcome those limitations in tef genetic gain information with objective of;

- To estimate the amount of genetic gain made in grain yield potential of tef, and
- To assess the changes brought about by genetic improvement on associated agronomic traits

II. Materials and Methods

The experiment were conducted at Debre-Zeit and Melkassa Agricultural Research Centre in the field in the 2012 main cropping season under rain fed conditions on two soil types: DebreZeit Black Soil (Vertisol) and MelkassaLightSoil (Andosol). In both experiments, 33 improved tef varieties that were successively released between 1970 and 2012 were used. Of these varieties 19varieties were released from Debre-Zeit Agricultural Research Center and the remaining 5, 3, 2, 2, 1 and 1 varieties were released from, Sirinka, Adet, Holleta, Bako, Melkassa and Areka Agricultural Research Centers respectively (Kebebew*et al*., 2013).

The experiment was conducted using randomized complete block design (RCBD) with three replications. Each plot had six rows of 3 m long and 1.2 m width (3.6 m^2) each with 0.2 m of row spacing. The distance between blocks and the spacing between plots were 1.5 and 1m, respectively. Seed rate was 1.5 g per row on the basis of 25 kg/ha recommended rate. Fertilizers were applied at both site at the recommended nutrient rate of 100 kg/ha N and 100 kg/ha DAP respectively. All other pre and post management practices were applied in accordance with the recommendations made for the crop. Data were collected from the four middle rows.

Days from sowing to 50% of the plants emerged in a plot,Days from sowing to 50% anthesis and from sowing to 50% maturity were determined. Plant height in centimeters was measured from the base of the plant to the tip of the panicle on the primary tiller of 10 randomly selected plants per plot. Panicle length of the central tillers in centimeters was measured as the average length of the panicle from the node where the first panicle branch starts to the tip of the central tiller of 10 randomly selected plants per plot. Panicle weight in milligrams was determined as the average weight of the central panicle of 10 randomly selected plants per plot.Lodging index was recorded using the method of Caldicott and Nuttall (1979). The angle of leaning was scored on a 0-5 scale where "0" stands for completely upright plants and "5" stands for completely lodged (flat on the ground) plants. The severity for each score was recorded as the percentage of the entire plot. Then, the lodging index was obtained as the average of the product sum of each degree of lodging and the corresponding severity percent.

Table 1 : Description of the experimental released tef varieties

No.	Variety	Year of release	Breeder /maintainer	Plant height (cm)	Days to mature	On-station yield (t/ha)	On farm yield (t/ha)
1	DZ-01-99 (Asgori)	1970	DZARC	53-100	80-130	2.2-2.8	1.8-2.2
2	DZ-01-196 (Magna)	1970	DZARC	53-115	80-113	1.8-2.4	1.6-2.0
3	DZ-01-354 (Enatite)	1970	DZARC	50-117	85-100	2.4-3.2	2.0-2.4
4	DZ-01-787 (Wellenkomi)	1978	DZARC	50-110	90-130	2.4-3.0	2.0-2.4
5	DZ-Cr-44 (Menagesha)	1982	DZARC	85-110	95-140	1.8-2.4	1.8-2.2
6	DZ-Cr-82 (Melko)	1982	DZARC	96-112	112-119	1.8-2.4	1.6-2.0
7	DZ-Cr-37 (Tsedey)	1984	DZARC	67-92	82-90	1.8-2.5	1.4-2.2
8	DZ-Cr-255 (Gibe)	1993	DZARC	63-116	114-126	2.0-2.6	1.6-2.2
9	DZ-01-974 (Dukem)	1995	DZARC	84-132	76-138	2.4-3.4	2.0-2.7
10	DZ-Cr-358 (Ziquala)	1995	DZARC	70-109	85-137	2.4-3.4	2.0-2.7
11	DZ-01-2053(Holetta Key)	1998	HARC	---	---	---	---
12	DZ-01-1278(Ambo Toke)	1999	HARC	---	---	---	---
13	DZ-01-1281 (Gerado)	2002	DZARC	83-100	73-95	1.7-2.4	1.6-2.2
14	DZ-01-1285 (Koye)	2002	DZARC	80-92	104-118	1.7-2.4	1.6-2.2
15	DZ-01-1681 (KeyTena)	2002	DZARC	74-85	84-93	1.7-2.4	1.6-2.2
16	DZ-01-2054(Gola)	2003	SARC	---	---	---	---
17	Ajora (PGRC/E 205396)	2004	ArARC	---	---	---	---
18	DZ-01-899 (DegaTef)	2005	DZARC	46-68	118-137	1.5-2.2	1.6-2.0
19	DZ-Cr-2675 (Chefe)	2005	DZARC	47-91	112-123	1.5-2.4	1.4-2.2
20	DZ-01-1868(Yilmana)	2005	AARC	---	---	---	---
21	DZ-01-2423 (Dima)	2005	AARC	---	---	---	---
22	DZ-01-146 (Genete)	2005	SARC	---	---	---	---
23	DZ-01-1821 (Zobel)	2005	SARC	---	---	---	---
24	HO-Cr-136 (Amarach)	2006	DZARC	67-81	63-87	1.8-2.5	1.4-2.2
25	DZ-Cr-387 RIL 355 (Quncho)	2006	DZARC	72-104	86-151	2.0-3.2	1.8-2.6
26	DZ-01-1880 (Guduru)	2006	BARC	---	---	---	---
27	Mechare(Acc.205953)	2007	SARC	---	---	---	---
28	DZ-Cr-387 RIL127 (Gemechis)	2007	MARC	---	---	---	---
29	DZ-01-3186 (Etsub)	2008	AARC	---	---	---	---
30	Kena(23-tafi-adi-72)	2008	BARC	---	---	---	---
31	DZ-Cr-285 RIL 295 (Simada)	2009	DZARC	65-90	75-90	1.9-2.8	1.6-2.5
32	Laketch -RIL273	2009	SARC	---	---	---	---
33	DZ-Cr-409 RIL50d (Boset)	2012	DZARC	---	---	---	---

Source: Kebebewet al. (2013), and MoA (2012)
 # = Abbreviations: AARC = Adet Agricultural Research Center, ArARC = Areka Agricultural Research Center, BARC = Bako Agricultural Research Center, DZARC= DebreZeit Agricultural Research Center, HARC = Holleta Agricultural Research Center, MARC = Melkassa Agricultural Research Center and.
SARC= Sirinka Agricultural Research Center,

Yield per Panicle was determined as the average grain weight obtained from the panicle of 10 randomly selected plants per plot and 100-kernel weight in milligrams was determined from dried samples of 100 grains.

Biomass yield was taken from all plants in the 3.6 m^2 of each plot and weighed as grams of biomass yield and converted to kg/ha. Grain yield was determined by threshing all plants in the 3.6 m^2 and expressed as kg/ha. Harvest index was calculated as

the ratio of grain yield to biomass yield. Biomass production rate was calculated as the ratio of biomass yield to days to physiological maturity and expressed as kg ha^{-1} day^{-1}. Grain yield per day was calculated as the ratio of grain yield to days to physiological maturity and expressed as kg ha^{-1} day^{-1}

All measured variables were subjected to analysis of variance procedures to assess differences among varieties. The homogeneity of error mean squares between the two locations were tested by F-test on variance ratio and combined analyses of variance were performed for the traits whose error mean squares were homogenous using PROC GLM procedure of SAS (SAS institute, 2002).Error variances were heterogeneous between the two locations for days to panicle emergence, days to maturity, plant height, lodging index, panicle weight, grain yield per day and biomass production rate. Hence, log-transformation of these traits was performed to remove heterogeneity according to Gomez and Gomez (1984). But, transformation could not stabilize error variances for the two locations for all the above traits. As a result, separate analysis of variances was done for these seven yield variables. Analysis of variance was carried out following the standard procedure given by Gomez & Gomez (1984). Mean separation was accomplished using Duncan's multiple range test (DMRT).

The annual rate of gain in grain yield potential and changes produced on agronomic traits were estimated by regressing the mean value of each character for each variety against the year of release for that variety using PROC REG procedure (SAS institute, 2002).The relative annual gain achieved over the last 42 years (1970 - 2012) was determined as a ratio of genetic gain to the corresponding mean value of oldest variety and expressed as percentage. Determination of correlation coefficients between grain yield and its components were computed using means of each variety. Pearson correlation coefficients among all characters were made using means of each variety, PROC CORR in SAS. Stepwise regression analysis was carried out on the varietal mean using PROC STEPWISE in SAS to determine those traits that contributed much for yield variation among varieties.

III. Results

a) Grain yield potential

The combined analysis of variance across the two locations revealed highly significant ($P \leq 0.01$) difference between locations and among varieties for grain yield, but there was no significant variety x location interaction (Table 2). The average grain yield of tef varieties was 4191 kg ha^{-1}, which ranged from 3094kg ha^{-1} for the variety released in 1982 (DZ-Cr-44) to 4934 kg ha^{-1} for the variety released in 2012 (DZ-Cr-409) (Table 4). The recently released variety Boset (DZ-Cr-409) was the first best yielder among the 33 varieties, but the difference was not significantly ($P \leq 0.05$) higher than ten varieties (DZ-Cr-387, Laketch (RIL273), DZ-01-2423, DZ-Cr-387, DZ-01-1868, DZ-01-1821, DZ-01-3186, Mechare (Acc. 205953), DZ-Cr-285 and DZ-01-1281) (Table 4). As indicated in Table 5, the superiority of the higher yielder variety, DZ-Cr-409 represents 1099 kg ha^{-1} or 28.65 % increment over the average of the first four older varieties (DZ-01-99, DZ-01-196, DZ-01-354 and DZ-01-787).

Mean grain yields of varieties released in1984, 1993, 1995, 2002, 2003, 2004, 2005, 2006, 2007, 2008, 2009 and 2012 exceeded that of the average of the first released four older varieties by 424.52 (11.07%), 501.12 (13.06%), 511.47 (13.33%), 427.39 (11.14%), 308.72 (8.05%), 364.62 (9.51%), 578.80 (15.09%), 529.49 (13.80%), 831.47 (21.68%), 224.12 (5.84%), 727.92 (18.98%) and 1098.82 kg ha^{-1} (28.65%), respectively. However, varieties released during 1982, 1998 and 1999s had decreased yield by -686.23 (-17.89 %), -250.18 (-6.52%) and -295.98 (-7.72%), respectively as compared with the average of four older varieties (Table 5).

Table 2 : Mean squares from the combined analysis of variance for grain yield and other traits of tef varieties evaluated over two test location (Debre Zeit and Melkassa) in 2012

Trait€	Location (1)a	Varieties (32)	Location x Varieties (32)	Error (128)	Mean	CV (%)	R^2
DSE	0.51^{ns}	1.51^{**}	0.65^{ns}	0.68	7.63	10.78	0.47
PL	116.79^{**}	62.59^{**}	12.78^{**}	5.02	44.46	5.04	0.80
YPP	0.31^{**}	0.28^{**}	0.12^{**}	0.04	1.06	19.16	0.72
HSW	0.001^{*}	0.0001^{ns}	0.0001^{ns}	0.0001	0.03	28.13	0.50
GYPha	22749171.60^{**}	1136443.24^{**}	133777.32^{ns}	148968.16	4190.58	9.21	0.77
BYPha	54703673.40^{**}	19138991.60^{**}	6269598.20^{**}	2605702.00	16281.00	9.91	0.72
HI	300.88^{**}	6.47^{**}	7.24^{**}	2.53	25.84	6.16	0.70

[a]Numbers in parenthesis represent degrees of freedom.

***, *, ns= Significant at $P \leq 0.01$, significant at $P \leq 0.05$ and non significant respectively.*

€ = Abbreviations, DSE= days to seedling emergence, PL= panicle length, YPP = yield panicle^{-1}, HSW = hundred seed weight (g), index, GYPha = grain yield per hectare (kg ha^{-1}), BYPha = biomass yield per hectare (kg ha^{-1}), HI = harvest index,

Table 3 : Mean squares from the separate analysis of variance for grain yield and related traits of tef varieties evaluated at DebreZeit and Melkassa during the 2012 main season

Trait€	Source of variation									
	DebreZeit					Melkassa				
	Variety[a] (32)	Error (64)	Mean	CV (%)	R^2	Variety (32)	Error (64)	Mean	CV (%)	R^2
DPE	7.58**	0.87	44.26	2.11	0.82	21.08**	7.57	40.80	6.75	0.59
DM	124.75**	1.08	98.33	1.06	0.98	72.50**	34.56	81.88	7.18	0.51
PH	125.38**	15.78	105.57	3.76	0.81	278.48**	36.92	111.99	5.43	0.79
LI	323.50**	75.14	66.00	13.13	0.69	0.07**	0.02	0.63	23.64	0.63
PW	0.27**	0.09	1.58	19.48	0.59	0.16**	0.05	1.54	14.09	0.65
GYPD	104.44**	14.78	46.30	8.30	0.78	119.20**	38.45	47.39	13.08	0.61
BPR	1737.88**	236.51	171.87	8.95	0.79	2589.59**	758.63	193.96	14.20	0.63

[a] = *Numbers in parenthesis represent degrees of freedom*

***, *, ns= Significant at P ≤ 0.01, significant at P ≤ 0.05 and non significant respectively;*

€ = *Abbreviations; DPE= days to panicle emergence, DM= days to maturity, PW = panicle weight, LI = lodging PH = plant height (cm), GYPD= grain yield per day (kg ha^{-1} day^{-1}) and BPR = biomass production rate (kg ha^{-1} day^{-1}).*

Table 4 : Mean grain yield (GYPha) in kg ha^{-1}, biomass yield (BYPha) in kg ha^{-1}, harvest index (HI) in %, grain yield per panicle (YPP) in mg, hundred seed weight (HSW) in mg, days to seedling emergency (DSE) and panicle length (PL) of tef varieties averaged over the two locations.

Varieties	GYPha	BYPha	HI	YPP	HSW	DSE	PL
DZ-01-99	3667.39^{ghij}	16805.56^{abcd}	24.37^{g}	0.79^{fgh}	0.03	7.83^{abcde}	42.48^{fgh}
DZ-01-196	3787.70^{efghij}	14138.89^{def}	28.59^{abcde}	0.80^{fgh}	0.03	8.17^{abc}	47.45^{bcd}
DZ-01-354	4090.95^{defgh}	17500.00^{abc}	26.27^{bcdefg}	1.40^{ab}	0.03	7.50^{abcde}	44.58^{cdefg}
DZ-01-787	3796.56^{efghi}	13944.44^{def}	29.30^{abc}	0.73^{gh}	0.04	6.83^{de}	44.68^{cdefg}
DZ-Cr-44	3093.84^{k}	12250.00^{f}	26.67^{bcdefg}	0.70^{gh}	0.03	7.17^{bcde}	43.5^{efg}
DZ-Cr-82	3204.95^{jk}	12027.78^{f}	26.54^{bcdefg}	0.70^{gh}	0.03	7.00^{cde}	42.50^{fgh}
DZ-Cr-37	4260.14^{cdef}	17875.00^{ab}	25.06^{fg}	0.87^{defgh}	0.03	7.17^{bcde}	39.42^{hi}
DZ-Cr-255	4336.75^{cd}	16472.22^{abcde}	29.38^{ab}	1.33^{ab}	0.04	7.67^{abcde}	45.50^{cdefg}
DZ-01-974	4387.33^{bcd}	17750.00^{ab}	27.92^{abcdef}	1.47^{ab}	0.03	7.00^{cde}	45.41^{cdefg}
DZ-Cr-358	4306.92^{cdef}	16250.00^{bcde}	28.23^{abcdef}	1.23^{abcde}	0.03	7.67^{abcde}	46.08^{cdef}
DZ-01-2053	3585.45^{hij}	13750.00^{ef}	28.28^{abcdef}	0.61^{h}	0.03	8.33^{ab}	37.32^{i}
DZ-01-1278	3539.70^{ijk}	14194.44^{def}	27.72^{abcdef}	0.87^{defgh}	0.04	8.00^{abcd}	44.99^{cdefg}
DZ-01-1281	4424.36^{abcd}	17250.06^{abcdef}	25.75^{abcdefg}	1.22^{abcd}	0.03	7.50^{abcde}	45.85^{cdef}
DZ-01-1285	4294.50^{cdef}	16680.56^{abcdefg}	25.80^{abcdefg}	1.27^{abc}	0.04	7.83^{abcde}	44.09^{defg}
DZ-01-1681	4070.28^{defgh}	15402.89^{defghi}	26.50^{abcdef}	0.79^{ij}	0.03	8.50^{a}	44.24^{defg}
DZ-01-2054	4144.33^{cdefg}	15077.83^{fghi}	27.90^{a}	1.03^{cdefghi}	0.04	8.17^{abc}	45.75^{cdef}
Ajora (PGRC/E 205396)	4200.33^{cdef}	15455.50^{defghi}	27.30^{ab}	0.87^{fghij}	0.03	7.33^{abcde}	43.85^{defg}
DZ-01-899	4281.25^{cdef}	16333.39^{bcdefg}	26.47^{abcdef}	1.03^{cdefghi}	0.04	7.83^{abcde}	46.96^{bcde}
DZ-Cr-2675	4275.11^{cdef}	15855.50^{cdefgh}	27.02^{abc}	1.10^{abcdefg}	0.03	7.67^{abcde}	44.87^{cdefg}
DZ-01-1868	4520.89^{abcd}	17958.50^{abc}	25.10^{bcdefg}	0.96^{defghij}	0.04	7.33^{abcde}	44.71^{cdefg}
DZ-01-2423	4574.20^{abcd}	17500.00^{abcd}	26.24^{abcdef}	1.12^{abcdef}	0.04	7.50^{abcde}	42.00^{gh}
DZ-01-146	4327.81^{cde}	16283.50^{bcdefg}	26.60^{abcdef}	1.18^{abcde}	0.03	8.17^{abc}	47.95^{bc}

DZ-01-1821	4507.59^{abcd}	18125.06^{ab}	24.82^{cdefg}	1.23^{abcd}	0.04	8.00^{abcd}	43.84^{defg}
HO-Cr-136	4283.00^{cdef}	17327.83^{abcde}	24.80^{cdefg}	1.00^{cdefghij}	0.03	8.17^{abc}	38.55^{i}
DZ-Cr-387 RIL 355	4549.86^{abcd}	18439.00^{ab}	24.70^{efg}	1.33^{ab}	0.03	6.67^{e}	49.97^{ab}
DZ-01-1880	4262.56^{cdef}	16833.23^{abcdefg}	25.30^{bcdefg}	1.12^{abcdef}	0.03	7.17^{bcde}	51.25^{a}
Mechare (Acc.205953)	4451.61^{abcd}	17697.33^{abc}	25.20^{bcdefg}	1.37^{a}	0.03	7.33^{abcde}	47.87^{bc}
DZ-Cr-387	4882.64^{ab}	18180.67^{ab}	26.90^{abcde}	1.35^{a}	0.03	6.83^{de}	45.50^{cdefg}
DZ-01-3186	4485.50^{abcd}	17749.94^{abc}	25.31^{bcdefg}	1.25^{abc}	0.03	8.17^{abc}	47.42^{bcd}
Kena (23-tafi-adi-72)	3634.08^{hij}	13938.94^{hijk}	25.97^{abcdefg}	0.80^{hij}	0.04	8.17^{abc}	43.49^{efg}
DZ-Cr-385 RIL 295	4449.44^{abcd}	18241.78^{ab}	24.42^{fg}	1.12^{abcdef}	0.03	7.00^{cde}	37.79^{i}
Laketch - RIL273	4677.61^{abc}	18912.83^{a}	24.70^{defg}	1.38^{a}	0.03	7.83^{abcde}	47.19^{bcd}
DZ-Cr-409	4934.44^{a}	18541.56^{ab}	27.01^{abcd}	1.22^{abcd}	0.04	8.17^{abc}	40.00^{hi}
Mean	4190.58	16280.96	25.84	1.06	0.03	7.63	44.46
CV (%)	9.21	9.91	6.16	19.16	28.10	10.78	5.04
R^2	0.77	0.72	0.70	0.72	0.50	0.47	0.80

Means followed by the same letter with in a column are not significantly different from each other at P≤ 0.05 according to Duncan's Multiple Range Test, [x]=Abbreviations, refer to Table 2

Table 5 : Trend in genetic progress in grain yield potential of tef varieties released in 1978, 1982, 1984, 1993, 1995, 1998, 1999, 2002, 2003, 2004, 2005, 2006, 2007, 2008, 2009 and 2012 over the average of the first three oldest varieties (DZ-01-99, DZ-01-196 and DZ-01-354) released in the 1970's

Varieties	Year of release	Mean grain yield	Increment over average of the first four older varieties (1970s)	
		kg ha^{-1}	kg ha^{-1}	%
DZ-01-99	1970			
DZ-01-196	1970	3848.68	-----	------
DZ-01-354	1970			
DZ-01-787	1978	3796.56	-52.12	-1.35
DZ-Cr-44	1982	3149.45	-699.23	-18.17
DZ-Cr-82	1982			
DZ-Cr-37	1984	4260.2	411.52	10.69
DZ-Cr-255	1993	4336.8	488.12	12.68
DZ-01-974	1995	4347.15	498.47	12.95
DZ-Cr-358	1995			
DZ-01-2053	1998	3585.5	-263.18	-6.84
DZ-01-1278	1999	3539.7	-308.98	-8.03
DZ-01-1281	2002			
DZ-01-1285	2002	4263.07	414.39	10.77
DZ-01-1681	2002			
DZ-01-2054	2003	4144.4	295.72	7.68
Ajora (PGRC/E 205396)	2004	4200.3	351.62	9.14

DZ-01-899	2005	4414.48	565.8	14.7
DZ-Cr-2675	2005			
DZ-01-1868	2005			
DZ-01-2423	2005			
DZ-01-146	2005			
DZ-01-1821	2005			
HO-Cr-136	2006	4365.17	516.49	13.42
DZ-Cr-387 RIL 355	2006			
DZ-01-1880	2006			
Mechare (Acc.205953)	2007	4667.15	818.47	21.27
DZ-Cr-387	2007			
DZ-01-3186	2008	4059.80	211.12	5.49
Kena (23-tafi-adi-72)	2008			
DZ-Cr-285 RIL 295	2009	4563.60	714.92	18.58
Laketch - RIL273	2009			
DZ-Cr-409	2012	4934.50	1085.82	28.21

The average rate of increase in yield potential per year of release estimated from the slope of the graph (Figure 1) was 21.53 kg ha^{-1} year^{-1} and it was significantly different from zero ($P \leq 0.05$) (Table 6). There was no indication of a yield potential plateau in tef over the period studied (Table 5).

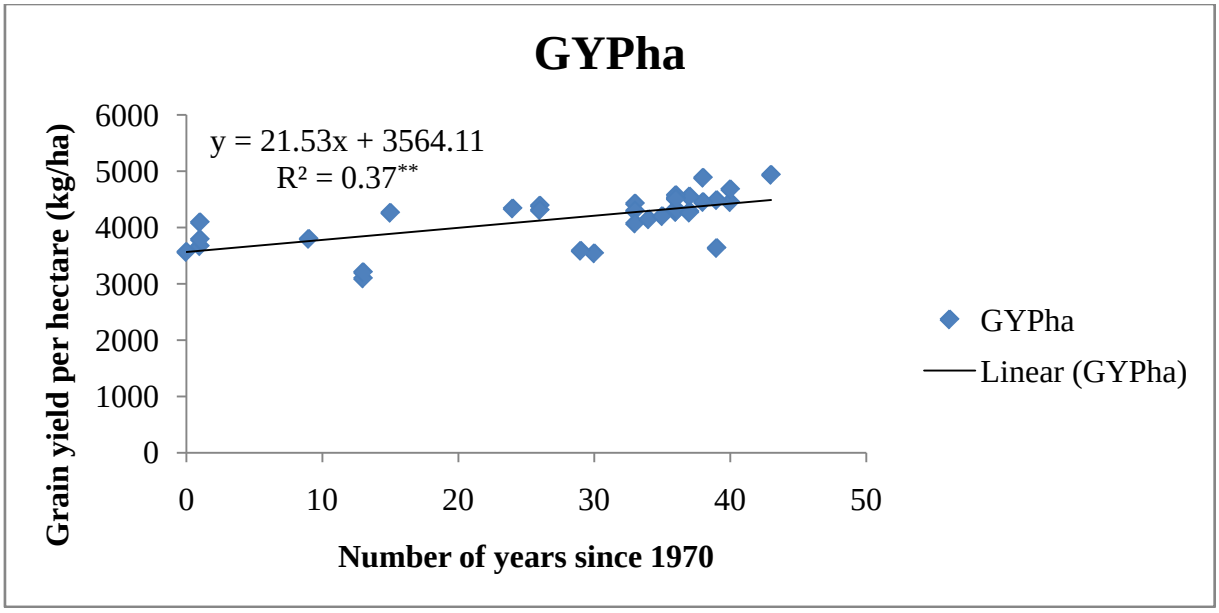

Figure 1 : Relationship between Mean Grain yield of 33 tef varieties and the year of release expressed as number of years since 1970

Table 6 : Mean values, coefficient of determination (R^2), regression coefficient (b) and intercept for various traits from linear regression of the mean value of each trait for each tef variety against the year of variety release since 1970 across location

Traits[x]	Mean	R^2	b	Intercept
DSE	7.63	0.03	0.01	7.44
PL	44.46	0.01	0.02	43.73
YPP	1.06	0.19	0.01*	0.84
HSW	0.03	0.03	0.0001	0.03
GYPha	4190.60	0.37	21.53**	3564.10
BYPha	16281.11	0.26	73.74**	14136.11
HI	25.84	0.04	0.02	25.36

** & ** = Significant at P ≤ 0.05 and P ≤ 0. 01, respectively,*

[x]=Abbreviations, refer to Table 2

b) Biomass Yield, Harvest Index and Plant Height

The combined analyses of variance across locations, depicted significant (P≤0.05) effects of location x variety interaction, between locations and among varieties for biomass yield (Table 2). When averaged over the two sites, variety, Laketch-RIL273 was significantly (P ≤ 0.05) higher than 15 varieties such as DZ-Cr-44, DZ-Cr-82, DZ-01-2053, DZ-01-1278, and Kena (23-tafi-adi-72), while this was not significantly different from 18 of the varieties including DZ-Cr-409, DZ-Cr-387 RIL 355, DZ-Cr-285 RIL 295, and DZ-Cr-387 (Table 4). Mean biomass yield of all tef varieties, averaged across locations was 16281 kg ha^{-1} (Table 2). The linear regression of biomass yield of tef variety means on year of variety release revealed a highly significant (P≤0.01) trend of increase over the period studied (Table 6). Accordingly, biomass yield increased by 73.74 kg ha^{-1} $year^{-1}$ (Figure 2).

Unlike grain yield, the combined analysis of variance for harvest index revealed highly significant (P≤0.01) location x variety interaction, between locations and among varieties (Table 2). Mean harvest index of the varieties was estimated to be about 26% (0.26) (Table 4). Linear regression coefficient indicated that harvest index for the period studied was 0.02 which is not significantly (P≤0.05) different from zero (Table 6).

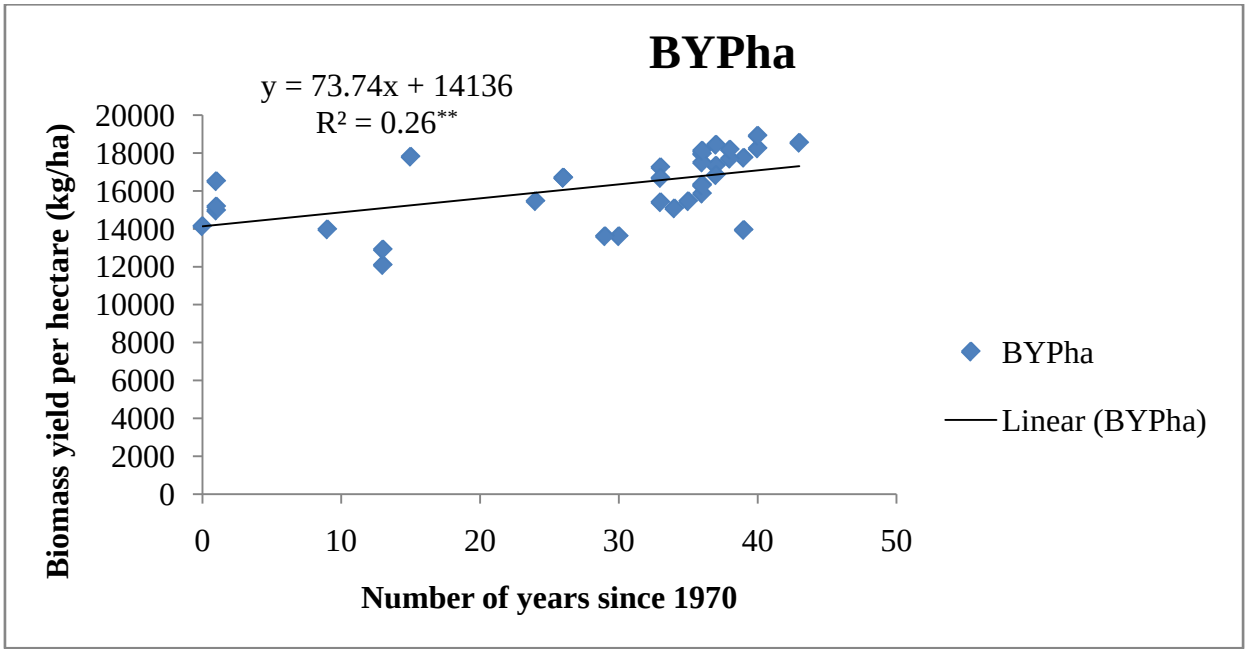

Figure 2 : Relationship between Mean Biomass yield of 33 tef varieties and the year of release expressed as number of years since 1970

Plant height was treated separately because mean squares of error for this trait were heterogeneous for the two locations. Accordingly, there was highly significant (P≤ 0.01) difference observed among varieties at both locations. Mean plant height of tef varieties was 105.57 cm at DebreZeit and 111.99 cm at Melkassa (Table 3). At both locations the same variety DZ-01-1880 was relatively tallest plant height than all the other varieties (Table 8 and 9). The regression of variety means against year of release at both locations was 0.02 and 0.04 cm year^{-1} at Debre-Zeit and Melkassa respectively, and this was not significantly different from zero (Table 7).

c) Yield Attributes

From the combined analysis of variance over the two test locations, variety × location interaction revealed significant (P≤0.05) effects on yield panicle $^{-1}$. The mean yield panicle^{-1} of varieties across location was estimated to be 1.06 mg (Table 2). The varieties Laketch-RIL273, Mechare (Acc.205953), DZ-Cr-387 and DZ-Cr-387 RIL 355 produced higher yield panicles^{-1}(Table 4). The linear regression of yield panicle^{-1} of tef variety means on the year of variety release revealed significant (P≤0.05) trend of increase over the period studied (0.01 mg year^{-1})(Table 6).

Panicle weight was highly significant (P≤0.01) differences among varieties both at DebreZeit, and Melkassa. Mean Panicle weight of the tef varieties represented in this study was 1.58 mg at DebreZeit and 1.54 mg at Melkassa (Table 3). At DebreZeit, the variety Laketch-RIL273 gave the heaviest panicle of all the test varieties (Table 8). At Melkassa, the variety DZ-Cr-387 (Gemechis) produced a heavier panicle weight than all other varieties, while the variety DZ-01-2053 produced the lightest panicle that was significantly (p≤0.05) different from that of all the other varieties used in the study (Table 9). Unlike for grain yield, significant (P≤0.05) genotype × location interaction was found for panicle length. Mean panicle length of the varieties averaged over the two locations was 44.46 cm (Table 2). The variety DZ-01-1880 showed the longest mean panicle length which was significantly (P≤0.05) different that of all the other varieties represented in the study except DZ-Cr-387 RIL 355(Table 4).

The linear regression analysis showed that the regression coefficient for panicle weight for the period studied was 0.01 at both location,which is significantly (P≤0.05) different from zero (Table 7). Unlike that of panicle weight, the linear regression of panicle length for the studied period was 0.02. But it is not significantly different from zero (Table 6).

Lodging indexwas highly significant (P≤0.01) differences among genotypes at both locations. The mean lodging indices were 66 and 63 at DebreZeit and Melkassa, respectively (Table 3). At DebreZeit, the variety DZ-01-1880 lodged relativelylower than all the other varieties(Table 10).At Melkassa, the variety Laketch-RIL273 showed relatively lower lodging index than all the other varieties (Table 9). The linear regression showed a slight but not significant (P≤0.05) decreasing trend over the 42 years period (Table 7).

d) Phenologic Traits

The combined analysis of variance over locations revealed that there were no significant (P≤0.05) effects of locations and genotype×location interaction on days to seedling emergence, while, there was highly significant (P≤0.01) differences among varieties in days to seedling emergence. Mean of days to seedling emergence of all varieties across the location represented in the trial was 7.63 days (Table 2). The variety DZ-Cr-387 RIL 355 was the earliest to emerge, though it was not significantly (P≤0.05) different from some other varieties (Table 4). Linear regression analysis showed that number of days to seedling emergence in modern varieties increased but non-significantl (Table 6).

Days to panicle emergence and days to maturity were highly significant (P≤0.01) differences among genotypes at both locations. Mean days to panicle emergence of all varieties were 44.26 and 40.80 at DebreZeit and Melkassa respectively (Table 3). At both locations the same variety, variety DZ-Cr-285 had the earliest panicle emergence(Table 8 and 9). Mean of days to maturity of varieties was 98.33 days at DebreZeit and 81.88 days at Melkassa (Table 3). At DebreZeit, the variety DZ-Cr-285 RIL 295 reached physiological maturity earlier (Table 8). At Melkassa, the variety DZ-01-2053 reached maturity earlier than the other varieties (Table 9). Regression analysis of number of days to panicle emergency and days to maturity at both locations showed negative regression coefficient, which was not significantly different from zero (Table 7).

e) Productivity Traits

There was no significant variety × location interaction and among varieties difference for hundred seed weight. It could be seen that hundred seed weight of modern tef varieties was not significantly different from that of the older varieties (Table 2). The linear regression depicted no significant (P≤0.05) linear relationship to cultivar age (Table 6).

There were highly significant (P≤0.01) differences among varieties in both biomass production rate and grain yield per day both at DebreZeit and Melkassa (Table 3). At DebreZeit, the newly released improved varieties, DZ-Cr-409 (Boset) and DZ-Cr-285 (Simada) depicted relatively the highest grain yield per day and biological production rate, respectively (Table 8). At Melkassa, nevertheless, DZ-Cr-387 (Gemechis) and DZ-Cr-409 (Boset) in that order gave significantly highest grain yield per day and biological production rate compared to all of the other varieties (Table 9).

Linear regression coefficient showed a highly significant ($P \leq 0.01$) increase in grain yield per day and biomass production rate at both locations except biomass production rate at melkassa which is significantly ($P \leq 0.05$) different from zero (Table 7).

Table 7 : Estimates of mean values, coefficient of determination (R^2), regression coefficient (b) and intercept for various traits from linear regression of the mean value of each traits for each tef variety against the year of variety release since 1970 for each locations

Traits[x]	Location							
	Debre-Zeit				Melkassa			
	Mean	R^2	b	Intercept	Mean	R^2	b	Intercept
DPE	44.26	0.001	-0.004	44.37	40.80	0.0003	-0.004	40.91
DM	98.33	0.01	-0.04	99.45	81.88	0.0001	-0.004	81.89
PH	105.57	0.002	0.02	104.93	111.99	0.002	0.04	110.94
LI	66.03	0.01	-0.001	68.92	62.54	0.05	-0.28	70.56
PW	1.58	0.17	0.01*	1.28	1.54	0.18	0.01*	1.30
GYPD (kg/ha/day)	46.30	0.29	0.26**	38.88	47.39	0.25	0.25**	40.00
BPR (kg/ha/day)	171.87	0.21	0.88**	146.24	193.96	0.13	0.84*	169.48

[x]=*Abbreviations, refer to Table 3*

f) Association of Grain Yield with other Traits

Grain yield was highly significantly ($r = 0.93^{**}$) and positively correlated with biomass yield, whereas it was not significantly ($r = 0.05^{**}$) associated with harvest index and plant height ($r = 0.08^{**}$) (Table 10). Yield per panicle showed highly significant ($r = 0.79^{**}$) and positive association with grain yield (Table 10). Yield attributes such as panicle weight, panicle length and lodging index showed a non-significant association with grain yield of $r = 0.29$, $r = -0.02$, and $r = -0.13$, respectively (Table 10).Phonologic traits (day to seedling emergence, days to panicle emergence and days to maturity) were observed absence of association with grain yield (Table 10). Correlation of grain yield with hundred seed weight was not significant ($r = 0.14$) (Table 10). There was highly significant positive association of grain yield with biomass production rate ($r = 0.83$) and grain yield day^{-1} ($r = 0.90$) (Table 10).

Step wise regression analysis of mean grain yield (dependent variable) on selected yield components (independent variable) indicated that biomass yield and harvest index were the two most important yield components which accounted for 99.7% of the variation in grain yield (Table 11).

Table 8 : Mean value of days to panicle emergency, days to maturity, lodging index, panicle weight, plant height, grain yield per day and biomass production rate of tef varieties at DebreZeit

Varieties	Trait[€]						
	DPE	DM	LI	PW	PH	GYPD	BPR
DZ-01-99	44.00^{efghi}	93.00^{ij}	80^{abc}	1.33^{cdefg}	98.60^{fgh}	43.94^{cdef}	180.80^{abcd}
DZ-01-196	45.33^{bcdef}	105.00^{b}	55^{ghi}	1.20^{defg}	110.70^{abc}	38.19^{fgh}	134.66^{fg}
DZ-01-354	44.33^{defghi}	100.33^{c}	64^{bcdefgh}	1.90^{abc}	109.60^{abc}	45.76^{bcde}	174.48^{bcde}
DZ-01-787	45.67^{bcde}	104.33^{b}	65^{bcdefgh}	1.34^{cdefg}	105.40^{bcdef}	39.20^{efg}	133.79^{fg}
DZ-Cr-44	45.33^{bcdef}	99.00^{cde}	62^{defgh}	1.00^{g}	105.10^{bcdef}	33.00^{gh}	123.74^{g}
DZ-Cr-82	44.33^{defghi}	99.00^{cde}	62^{defgh}	1.13^{efg}	105.60^{bcdef}	32.06^{h}	121.66^{g}
DZ-Cr-37	42.00^{jkl}	89.67^{lm}	78^{abcd}	1.2d^{efg}	96.60^{ghi}	49.84^{abc}	199.43^{ab}
DZ-Cr-255	44.33^{defghi}	104.00^{b}	71^{abcdefg}	1.72^{abcdef}	108.10^{bcd}	46.51^{bcd}	158.39^{def}
DZ-01-974	43.33^{ghijk}	97.67^{defg}	66^{bcdefgh}	1.93^{abc}	108.60^{bcd}	50.72^{abc}	181.72^{abcd}
DZ-Cr-358	43.33^{ghijk}	98.00^{def}	65^{bcdefgh}	1.75^{abcd}	107.60^{bcd}	46.82^{bcd}	165.86^{cde}
DZ-01-2053	43.00^{hijk}	92.67^{jk}	85^{a}	1.12^{fg}	91.60^{i}	41.92^{def}	148.24^{efg}
DZ-01-1278	44.67^{cdefgh}	105.00^{b}	81^{ab}	1.20^{defg}	104.50^{bcdef}	37.31^{fgh}	135.19^{fg}

DZ-01-1281	43.67^{fghij}	94.67^{hi}	71^{abcdefg}	1.67^{abcdef}	104.20^{cdef}	49.41^{abcd}	185.23^{abcd}
DZ-01-1285	43.00^{hijk}	97.33^{efg}	74^{abcdef}	1.90^{abc}	104.90^{bcdef}	46.66^{bcd}	171.50^{bcde}
DZ-01-1681	43.00^{hijk}	91.33^{jkl}	84^{a}	1.23^{defg}	100.80^{defgh}	49.71^{abc}	180.12^{abcd}
DZ-01-2054	44.00^{efghi}	99.00^{cde}	60^{efgh}	1.82^{abcd}	108.80^{bc}	47.75^{bcd}	181.86^{abcd}
Ajora (PGRC/E 205396)	42.00^{jkl}	96.67^{fg}	75^{abcde}	1.69^{abcdef}	107.70^{bcd}	48.52^{abcd}	173.39^{bcde}
DZ-01-899	45.00^{bcdefg}	103.67^{b}	62^{defgh}	1.87^{abc}	103.80^{cdefg}	47.15^{bcd}	187.53^{abcd}
DZ-Cr-2675	44.00^{efghi}	99.33^{cd}	69^{abcdefg}	1.87^{abc}	107.30^{bcde}	48.50^{abcd}	182.11^{abcd}
DZ-01-1868	45.33^{bcdef}	104.00^{b}	63^{cdefgh}	1.35^{cdefg}	105.40^{bcdef}	46.58^{bcd}	180.29^{abcd}
DZ-01-2423	43.67^{fghij}	96.00^{gh}	61^{efgh}	1.52^{abcdefg}	106.30^{bcdef}	50.88^{abc}	192.42^{abc}
DZ-01-146	45.00^{bcdefg}	98.00^{def}	57^{fgh}	1.76^{abcd}	112.50^{ab}	48.37^{abcd}	167.23^{cde}
DZ-01-1821	45.00^{bcdefg}	105.00^{b}	51^{hi}	1.64^{abcdef}	111.60^{abc}	46.07^{bcde}	182.01^{abcd}
HO-Cr-136	42.67^{ijk}	89.00^{m}	77^{abcde}	1.20^{defg}	96.20^{hi}	50.86^{abc}	196.63^{abc}
DZ-Cr-387 RIL 355	48.67^{a}	94.67^{hi}	57^{fgh}	1.80^{abcd}	110.50^{abc}	51.65^{abc}	198.66^{ab}
DZ-01-1880	46.00^{bcd}	96.67^{fg}	40^{j}	1.73^{abcde}	116.90^{a}	46.84^{bcd}	177.24^{abcde}
Mechare (Acc.205953)	44.67^{cdefgh}	96.67^{fg}	63^{cdefgh}	1.97^{ab}	109.30^{abc}	49.91^{abc}	184.56^{abcd}
DZ-Cr-387	45.00^{bcdefg}	93.00^{ij}	70^{abcdefg}	1.90^{abc}	107.70^{bcd}	53.48^{ab}	194.44^{abc}
DZ-01-3186	45.00^{bcdefg}	105.00^{b}	55^{ghi}	1.77^{abcd}	112.30^{ab}	46.75^{bcd}	173.54^{bcde}
Kena (23-tafi-adi-72)	46.33^{bc}	121.00^{a}	51^{hi}	1.37^{bcdefg}	110.80^{abc}	34.05^{gh}	120.97^{g}
DZ-Cr-385 RIL 295	40.67^{k}	88.00^{m}	76^{abcde}	1.50^{abcdefg}	84.90^{j}	53.39^{ab}	206.11^{a}
Laketch - RIL273	46.67^{b}	97.33^{efg}	57^{fgh}	2.00^{a}	110.40^{abc}	50.18^{abc}	196.20^{abc}
DZ-Cr-409	41.67^{kl}	91.00^{l}	72^{abcdefg}	1.67^{abcdef}	99.50^{efgh}	55.83^{a}	181.62^{abcd}
Mean	44.26	98.33	0.66	1.58	105.57	46.3	171.87
CV (%)	2.11	1.06	13.13	19.48	3.76	8.3	8.95
R^2	0.82	0.98	0.69	0.59	0.81	0.78	0.79

Means with in a column followed by the same letter are not significantly different at P ≤ 0.05 according to Duncan's Multiple Range Test, € = Abbreviations: DPE= days to panicle emergence, DM= days to physiological maturity, PH = plant height, PW = panicle weight, LI = lodging index, GYPD= grain yield per day (Kg ha^{-1} day^{-1}) and BPR = biomass production rate (Kg ha^{-1} day^{-1}).

Table 9 : Mean value of days to panicle emergency, days to maturity, lodging index, panicle weight, plant height, grain yield per day and biomass production rate of tef varieties at Melkassa

Varieties	Trait€						
	DPE	DM	LI	PW	PH	GYPD	BPR
DZ-01-99	39.67^{cdefgh}	76.67^{ef}	78.33^{abcd}	1.20^{e}	100.40^{jkl}	42.95^{cdefgh}	173.56^{defgh}
DZ-01-196	42.67^{abcdefg}	84.00^{abcdef}	69.67^{abcdef}	1.58^{abcde}	116.60^{bcdefg}	42.94^{cdefgh}	186.03^{cdefgh}
DZ-01-354	40.33^{bcdefg}	82.67^{abcdef}	70.33^{abcdef}	1.43^{abcde}	111.40^{efghij}	43.07^{cdefgh}	196.95^{bcdefg}
DZ-01-787	44.00^{abcde}	90.33^{ab}	67.67^{abcdef}	1.40^{bcde}	114.80^{cdefgh}	38.83^{fgh}	154.99^{fgh}
DZ-Cr-44	40.00^{cdefgh}	80.33^{abcdef}	59.33^{bcdefgh}	1.30^{de}	117.30^{bcdef}	36.86^{gh}	149.97^{gh}
DZ-Cr-82	38.33^{fgh}	81.00^{abcdef}	52.67^{defgh}	1.33^{cde}	121.70^{abcde}	40.08^{efgh}	170.61^{defgh}

DZ-Cr-37	38.00^{gh}	76.00^{ef}	73.33^{abcde}	1.47^{abcde}	101.30^{ijkl}	53.59^{abc}	234.57^{abc}
DZ-Cr-255	41.67^{abcdefg}	87.33^{abcde}	55.00^{cdefgh}	1.30^{de}	105^{ghijk}	44.14^{bcdefgh}	166.61^{efgh}
DZ-01-974	45.67^{ab}	79.33^{abcdef}	55.00^{cdefgh}	1.53^{abcde}	125.30^{abc}	48.47^{bcdefg}	198.73^{bcdefg}
DZ-Cr-358	42.33^{abcdefg}	90.67^{a}	73.00^{abcde}	1.77^{abc}	114.60^{cdefgh}	44.42^{bcdefgh}	188.77^{bcdefgh}
DZ-01-2053	41.33^{abcdefg}	75.00^{f}	93.00^{a}	0.80^{f}	91^{lm}	43.84^{bcdefgh}	179.90^{cdefgh}
DZ-01-1278	38.00^{gh}	78.67^{bcdef}	61.00^{bcdefgh}	1.40^{bcde}	110^{efghij}	40.15^{efgh}	165.01^{efgh}
DZ-01-1281	40.67^{bcdefg}	85.00^{abcdef}	72.00^{abcde}	1.73^{abcd}	107^{fghij}	49.06^{bcdefg}	198.99^{bcdefg}
DZ-01-1285	39.67^{cdefgh}	81.67^{abcdef}	57.67^{cdefgh}	1.70^{abcd}	112.90^{defghi}	49.97^{abcdef}	205.95^{bcdef}
DZ-01-1681	38.67^{efgh}	77.33^{def}	79.33^{abcd}	1.35^{cde}	103.20^{hijk}	47.56^{bcdefg}	189.64^{bcdefgh}
DZ-01-2054	41.67^{abcdefg}	87.33^{abcde}	46.67^{efgh}	1.20^{e}	119.10^{abcdef}	40.80^{defgh}	139.18^{h}
Ajora (PGRC/E 205396)	37.67^{gh}	76.00^{ef}	69.00^{abcdef}	1.49^{abcde}	107.40^{fghij}	48.85^{bcdefg}	186.33^{cdefgh}
DZ-01-899	40.33^{bcdefg}	83.00^{abcdef}	50.67^{defgh}	1.36^{cde}	117.30^{bcdef}	44.34^{bcdefgh}	159.87^{fgh}
DZ-Cr-2675	40.00^{cdefgh}	79.00^{abcdef}	51.00^{defgh}	1.52^{abcde}	113.90^{cdefgh}	47.33^{bcdefg}	172.98^{defgh}
DZ-01-1868	40.67^{bcdefg}	83.00^{abcdef}	66.00^{abcdefg}	1.70^{abcd}	109.30^{fghij}	50.58^{abcdef}	207.96^{bcdef}
DZ-01-2423	39.33^{defgh}	76.67^{ef}	56.33^{cdefgh}	1.83^{ab}	109.10^{fghij}	55.68^{ab}	216.15^{abcde}
DZ-01-146	40.33^{bcdefg}	78.00^{cdef}	35.33^{h}	1.83^{ab}	115.50^{bcdefg}	50.88^{abcdef}	209.91^{bcdef}
DZ-01-1821	42.00^{abcdefg}	87.00^{abcde}	52.33^{defgh}	1.77^{abc}	112.50^{defghi}	48.16^{bcdefg}	197.19^{bcdefg}
HO-Cr-136	37.67^{gh}	76.33^{ef}	87.67^{ab}	1.67^{abcd}	94.70^{klm}	53.01^{abcd}	225.37^{abcd}
DZ-Cr-387 RIL 355	46.33^{a}	80.67^{abcdef}	77.67^{abcd}	1.83^{ab}	127.20^{ab}	52.19^{abcde}	224.47^{abcd}
DZ-01-1880	45.00^{abc}	88.67^{abcd}	41.67^{fgh}	1.53^{abcde}	130^{a}	45.24^{bcdefgh}	186.82^{cdefgh}
Mechare (Acc.205953)	40.00^{cdefgh}	82.00^{abcdef}	61.00^{bcdefgh}	1.77^{abc}	116.40^{bcdefg}	50.22^{abcdef}	216.68^{abcde}
DZ-Cr-387	39.00^{efgh}	78.67^{bcdef}	68.67^{abcdef}	1.87^{a}	113^{defghi}	61.56^{a}	234.75^{abc}
DZ-01-3186	44.00^{abcde}	86.67^{abcdef}	37.00^{gh}	1.67^{abcd}	119.20^{abcdef}	46.97^{bcdefgh}	199.81^{bcdefg}
Kena (23-tafi-adi-72)	43.67^{abcdef}	90.00^{ab}	55.33^{cdefgh}	1.43^{bcde}	116.30^{bcdefg}	34.96^{h}	147.00^{gh}
DZ-Cr-385 RIL 295	34.67^{h}	75.67^{ef}	84.33^{abc}	1.63^{abcd}	88.50^{m}	55.53^{ab}	242.49^{ab}
Laketch - RIL273	44.67^{abcd}	89.33^{abc}	33.00^{h}	1.70^{abcd}	124.30^{abcd}	50.06^{abcdef}	209.82^{bcdef}
DZ-Cr-409	38.33^{fgh}	78.00^{cdef}	72.67^{abcde}	1.64^{abcd}	109.40^{fghij}	61.50^{a}	263.65^{a}
Mean	40.8	81.88	0.63	1.54	111.99	47.39	193.96
CV (%)	6.75	7.18	23.64	14.09	5.43	13.08	14.20
R^2	0.59	0.51	0.63	0.65	0.79	0.61	0.63

Means with in a column followed by the same letter are not significantly different at P ≤ 0.05 according to Duncan's Multiple Range Test, € = Abbreviations: DPE= days to panicle emergence, DM= days to physiological maturity, PH = plant height, PW = panicle weight, LI = lodging index, GYPD= grain yield per day (Kg ha^{-1} day^{-1}) and BPR = biomass production rate (Kg ha^{-1} day^{-1}).

Table 10 : Estimate of simple correlation coefficient of different traits with grain yield (R_{GYPha}), year of release of the variety (R_{YoR}) and biomass yield (R_{BYPha}).

Traits	Correlation coefficient (R)		
	R_{GYPha}	R_{YoR}	R_{BYPha}
Days to seedling emergence	0.19	0.16	0.27
Days to panicle emergence	-0.06	0.001	-0.08

Days to Maturity	-0.04	-0.03	0.01
Plant height	0.08	0.05	0.03
Panicle length	-0.02	0.10	-0.04
Lodging index	-0.13	-0.20	-0.09
Panicle Weight	0.29	0.41	0.19
Yield / panicle	0.79**	0.43	0.78**
Hundred seed weight	0.14	0.18	0.12
Grain yield/ha	-------	0.61*	0.93**
Grain yield per day	0.90**	0.54*	0.86**
Biological yield	0.93**	0.51*	-------
Biomass production rate	0.83**	0.44*	0.91**
Harvest Index	0.05	0.20	-0.31

**, ** = Significant at P ≤ 0.05 and P ≤ 0.01, respectively*

Table 11 : Summary of selection from stepwise regression analysis of mean grain yield of tef as dependent variable on the other traits as independent variables

Independent variables	Constant	Regression coefficient (b)	R^2	Variation Inflation Factor
Biomass yield per hectare	-4017.7	0.26	0.872	0.002
Harvest index		156.52	0.125	3.82

All regression coefficients are significant at P ≤ 0.01

IV. Discussion

As indicated in Table 5, the superiority of the higher yielder variety, DZ-Cr-409 represents 1086 kg ha^{-1} or 28.21 % increment over the average of the first three older varieties (DZ-01-99, DZ-01-196 and DZ-01-354). Nearly similar trends of genetic progress were reported in different crops in different parts of the world. In tef at DebreZeit grain yield of the recently released cultivar, DZ-01–974, showed significantly (P ≤ 0.05) higher grain yield than all varieties tested in the trial (Yifru and Hailu, 2005). It exceeded the farmer's variety and DZ-01-354, which is the most popular and the first improved variety by 34.3% and 41.44% respectively. In winter wheat in UK, seed yield of newly released cultivars was found to be 27.6% greater than the older cultivars (Shearman *et al*., 2005). Likewise, Wondimu (2010) who worked on malt barley reported that an increment in seed yield of 1690 kg/ha (51%) and 1388 kg/ha (38%) of modern varieties over the farmers variety, Balami and the oldest improved variety, IAR/H/485 respectively. Consistent yield improvement was observed in different years as indicating in Table 5. This revealed that grain yield potential of tef has not attained a plateau in Ethiopia; thus, provide that an opportunity for breeders to further improve tef yield through the existing breeding strategy. In line with the present findings, Amsal (1994) in wheat and Wondimu (2010) in barley found no trends of a plateau.

The average rate of increase in grain yield was 21.53 kg ha^{-1} $year^{-1}$, and it was highly significantly (P ≤ 0.01) different from zero. This reveals that tef breeders have made considerable efforts over the last 42 years to improve the yields of tef in the country. Similar trends have been reported by Yifru and Hailu (2005) in tef with comparable genetic gains of 27.16 kg/ha (0.79%) per year of release. Likewise Amsal (1994) in durum wheat, Wondimu (2010) in barley and Demissew (2010) in soybean reported respective increases of 64, 27.16, 44.24, and 13.26 kg ha^{-1} $year^{-1}$ in grain yield potential of varieties over the year of release in Ethiopia.

An improved biomass yield, days to seedling emergency, panicle length, yield per panicle and harvest index were the characteristics of most of the modern tef genotypes. Regression analysis of these traits over year of cultivar release (since1970) showed significant and positive regression coefficients for biomass yield and yield per panicle (Table 6). These result simply that the tef improvement program has made substantial progress in improving these traits. Likewise, Yifru and Hailu (2005) in tef, Mihret (2012) in sorghum at both location (Melkassa and Mieso) found an increase of biomass yield of modern varieties. From the separate analysis days to panicle emergency, days to maturity and lodging index were decreased non-significantly. Panicle weights, plant height, grain yield per day and biomass production rate were increased with year of release. Out of these panicle weight, grain yield per day and biomass production rate were increased significantly (Table 7).Similar to the present study, Mihret (2012) in sorghum reported that an increased trend in biomass production rate and grain filling rate at Melkassa and Mieso. Likewise, Wonidmu (2010) in barley observed significant changes in the total grain sink filling rate with year of cultivar release. In contrary, Yifru and Hailu (2005) and Wondimu (2010) observed non-significant increases in biomass production rate in tef and food barley yield, respectively.

Examination of components of yield by a series of simple correlations indicated that grain yield was positively and highly significantly associated only with biomass yield($r = 0.93^{**}$), yield per panicle ($r = 0.79^{**}$), biomass production rate ($r = 0.83^{**}$) and grain yield per day ($r = 0.90^{**}$) (Table 10). Similarly, positive association of biomass yield, biomass production rate and grain yield per day with grain yield were also reported by Yifru and Hailu (2005) in tef, Mihret (2012) in sorghum and Wondimu (2010) in barley. The other traits have no positive and negative contribution on grain yield. This was supported the findings of Yifru and Hailu (2005) in tef. Similarly, Mihret (2012) found non-significant negative association for grain yield with days to flowering and day to maturity in sorghum. Step wise regression analysis of mean grain yield (dependent variable) on selected yield components (independent variable) indicated that biomass yield and harvest index were the two most important yield components which accounted for 99.7% of the variation in grain yield (Table 11). It is, therefore, concluded that genetic yield potential improvement of tef over the last 42 years has been associated mostly with a corresponding increase in biomass yield and harvest index. This is in agreement with the findings of Mihret (2012) in sorghum at Mieso and Wondimu (2010) in malt barley indicated that biomass yield and harvest index were the most important traits contributing to the variation in the improvement programs.

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Evaluation of the Safety of Whole Milk in Wolaita Zone, Southern Ethiopia

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Keywords: *safety, milk, producers, collectors.*

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Evaluation of the Safety of Whole Milk in Wolaita Zone, Southern Ethiopia

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Abstract- A study was conducted to evaluate the quality and compositions of whole raw cow milk from farm(producers handling levels) to table(collectors handling levels) in Wolaita Zone of Soddo Town and Kokate Kebele. A total of thirty milk samples were collected from 30 dairy farmers (producers) at Kokate kebele who sold their milk at Soddo town and twenty five whole milk samples were collected from 25 café's and hotels (collectors) at Soddo town using clean test tube. The major components(fat, lactose, SNF, protein, salt and water) and physical properties(density, temperature and freezing point) of milk samples were immediately analyzed using milk analyzer machine. The result showed that the mean content of lactose, SNF, protein, salt, density and Freezing point of milk samples at producers handling conditions (4.71± 0.08, 8.40±0.13, 3.04± 0.05, 0.73± 0.01, 27.29± 0.56 and 0.60± 0.01) were significantly higher than that of collectors levels (3.74±0.15, 6.73±0.26, 2.41±0.10, 0.58±0.02, 21.34±0.93 and 0.43±0.02) . On the other hand, water content and temperature level of milk samples at producers levels(1.56± 1.31 and 22.33± 0.29) were significantly lower than collectors levels(18.25±3.20 and 22.69±0.40). However, the fat content of milk samples was not significantly different at producers and collectors handling conditions, even though the mean fat level of milk samples at collectors handling was higher than producers handling conditions. Generally, the present result indicated that the quality of milk was higher at producers handling conditions than collectors handling levels.

Keywords: safety, milk, producers, collectors.

I. Introduction

Ethiopia has great potential for dairy development due to its large livestock population and the favorable climate for livestock. Milk and milk products contributes considerably to the household and national economy through income and employment generation. Thus, the dairy sector is one the potential livestock sectors that contributes to poverty alleviation and improves household nutrition in the country (Mohammed *et al*., 2004).

Milk is a very nutritious food that is rich in carbohydrates, proteins, fats, vitamins and minerals which is a major constituent of the diet and is considered essential to the health and well being of the community (Prejit-Nanu and Latha, 2007). The nutritional as well as economic value of milk is directly associated with its solids content. The higher the solids content, the better its nutritional value and the greater the milk product yields. According to O"Connor (1994), the average total protein, total solids, ash, casein and lactose content of milk ranges between 2.9-5 %, 10.5-14.5 %, 0.6-0.9 %, 2.9-5 % and 3.6-5.5 %, respectively. The constituents may vary with breed, type of feed, stage of lactation, season and age of the cow etc. Milk quality is the sum of physico-chemical, microbial and sensory attributes and these attributes may deteriorate by a number of factors such as adulteration, contamination during and after milking and the presence of udder infections (Esron *et al.,* 2005).

Yet hygienic quality control of milk and milk products in Ethiopia is not usually conducted on routine basis. Door-to-door raw milk delivery in the urban and periurban areas is commonly practiced with virtually no quality control at all levels (Godefay and Molla, 2000). Therefore, this study was conducted to support dairy development through evaluating the nutritional quality of whole raw cow milk at producers and collectors handling conditions.

II. Materials and Methods

a) Description of the study area and sampling techniques

The study was conducted at kokate kebele (rural area) and Wolaita Soddo town (urban area) of Wolayta zone, Southern Ethiopia. Wolaita Soddo town is located at a distance of 330km south of the capital, Addis Ababa where as kokate kebele is located at a distance of about 6km north of the capital, Wolayta Soddo. The kebele and town were purposefully selected due to high milk marketing and consumption activities in Soddo town and Kokate kebele is its main milk shed area.

A total of 30 whole raw cow milk samples were collected from 30 dairy farmers (producers) at Kokate kebele who sold their milk at Soddo town and another 25 whole raw cow milk samples were collected from 25 café's and hotels (collectors) at Soddo town using clean test tube. Milk samples were immediately analyzed for their important compositions using milk analyzer machine that was borrowed from Debre Zeit Agricultural Research Center. Therefore, fat%, density, lactose%, SNF%, protein%, water%, temperature, freezing point and salt were determined in both producers and collectors whole milk handling conditions.

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b) Data analysis

Independent sample T-test was employed for analyzing the collected data by using SPSS (version 16) software. Descriptive statistics such as mean and percentages were used to summarize data as required. Probability (P) value less than 0.05 was used to determine the level of significance.

III. Results and Discussion

Table 3.1 : Nutritional composition and physical properties of fresh cow milk samples under different actors

Parameters	Producers	Collectors
Fat (%)	4.12± 0.28 [a]	4.21± 0.34 [a]
Density(%)	27.29± 0.56 [a]	21.34±0.93 [b]
Lactose(%)	4.71± 0.08 [a]	3.74±0.15 [b]
SNF(%)	8.40± 0.13 [a]	6.73±0.26 [b]
Protein(%)	3.04± 0.05 [a]	2.41±0.10 [b]
Water(%)	1.56± 1.31 [b]	18.25±3.20 [a]
Temperature	22.33± 0.29 [b]	22.69±0.40 [a]
Freezing point	0.60± 0.01 [a]	0.43±0.02 [b]
Salt	0.73± 0.01 [a]	0.58±0.02 [b]

Reported values are the mean ±SE (n=3). Means with different letters in the same rows are significantly different (p<0.05).

A study on milk handling in different actors is most important to evaluate the milk safety and quality after milking of cows. The present study described the effect of nutritional composition, safety and quality of whole raw cow milk under different actors such as producers and collectors handling conditions was shown in Table 3.1. The mean fat content was lower in producers (4.12g/100g) handling conditions than that of collectors(4.21g/100g) handling conditions, but statistically the value was not significant. The finding in this study was in line with Jemila G. and Achenef M.(2012). However, the finding disagrees with the report of Cayot(1998) and Kontel (1999) who reported that fat content of the cow milk was 3.90 g/100g and 3.90g/100g respectively. This may be due to differences in breed and other confounding factors (Guetouache M.*etal*.,2014).The mean density of whole raw cow milk samples at producers (27.27 g/100g) handling conditions was significantly higher than that of collectors (21.34 g/100g) handling conditions. This may be due to more water is added to milk samples in case of collectors. However, the present finding at producers handling levels agrees with the findings of Jemila G. and Achenef M.,(2012).

In this study, the mean content of lactose was significantly affected in collectors handling condition. Higher content of lactose(4.71%) was observed in producers level than that of collectors(3.74%) levels. Lactose is the main carbohydrate of milk so it may be used as substrate during the fermentation of milk by lactic acid bacteria at collectors handling conditions. Lactose content at producers handling conditions in this finding is similar with finding of Cayot,1998 and Jemila G. and Achenef M.,(2012) but different at collectors levels.

The solid not fat(SNF) content in producers(8.40 g/100g) levels was higher than that of collectors(6.73 g/100g) handling conditions. This result is agrees with findings of Jemila G. and Achenef M.,(2012) at producers milk handling condition but not at collectors handling conditions. This indicates that whole raw milk may be added with water at collectors conditions.

The protein content of whole raw milk in producers and collectors handling conditions are show in Table3.1. The mean protein level of whole raw milk significantly higher at producer handling condition than collectors handling condition. This indicates that some amount of milk protein is loss in case collectors.

Table 3.1 shows that salt and freezing point of whole raw milk were higher at producers handling conditions than at collectors. On the other hand, the mean value of water and temperature of whole raw milk at collectors handling condition were significantly higher than that of producers handling condition. The mean fat content at both producer and collectors handling condition were not significantly different.

In conclusion, the present result demonstrated that compositions of whole raw cow milk were varies at producers and collectors handling levels. The study indicated that the quality of whole raw cow milk was higher at producers handling levels than collectors handling levels.

IV. Acknowledgements

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Scaling up Agroforestry Farming System in Malawi: A Case of Malawi Agroforestry Extension Project

By Kakhobwe C. M., Kamoto J. F., Njoloma J. P & Nicholas Ozor
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Abstract- The study examined the factors affecting agroforestry technology upscaling and identified gaps in scaling up approaches of agroforestry technologies. One hundred and sixty four farmers in Malawi Agroforestry Extension (MAFE) project districts of Mzimba, Ntcheu and Mangochi were interviewed. Logistic model was used in analyzing data from the study. Results show that farmers' extension access, perceived usefulness of agroforestry technology, main source of income, educational level of household head, and number of field plots were the main factors affecting the scaling up of the agroforestry technologies in the area. Among others, the study recommended that farmers and extension workers should be actively and jointly engaged in the design of agroforestry projects for effective upscaling and that agroforestry extension services should be promoted for farmers to perceive the usefulness of the technologies to enhance scaling up of the technology.

Keywords: *malawi agroforestry, soil fertility, scaling up of agroforestry.*

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Kakhobwe C. M. [α], Kamoto J. F. [σ], Njoloma J. P [ρ] & Nicholas Ozor [ω]

Abstract- The study examined the factors affecting agroforestry technology upscaling and identified gaps in scaling up approaches of agroforestry technologies. One hundred and sixty four farmers in Malawi Agroforestry Extension (MAFE) project districts of Mzimba, Ntcheu and Mangochi were interviewed. Logistic model was used in analyzing data from the study. Results show that farmers' extension access, perceived usefulness of agroforestry technology, main source of income, educational level of household head, and number of field plots were the main factors affecting the scaling up of the agroforestry technologies in the area. Among others, the study recommended that farmers and extension workers should be actively and jointly engaged in the design of agroforestry projects for effective upscaling and that agroforestry extension services should be promoted for farmers to perceive the usefulness of the technologies to enhance scaling up of the technology.

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I. Introduction

Malawi is an agro-based country, which depends mainly on maize as staple food. However, maize production faces many challenges especially declining soil fertility (Malawi Government, 2012). In trying to solve the problem, the Malawi Agroforestry Extension Project (MAFE) was implemented between 1992 and 2002. The project had numerous achievements during the implementation period but there is little continuation and scale up of the agroforestry activities in the project sites after the project phased out in 2002. Scaling up agroforestry technology is important because soils in Malawi lose nutrients at annual rates of not less than 40 kg of nitrogen (N), 6.6 kg phosphorus (P) and 33.2 kg potassium (K) per hectare (Makumba, 2003). In order to sustain crop production in Malawi, farmers mainly rely on inorganic fertilizers. However, its sustainability is becoming even more difficult to achieve because the inorganic fertilizers are becoming unaffordable to most smallholder farmers due to the rise in prices (Kwesiga *et al*., 2003). This high cost of fertilizers has resulted in low application rates of less than 10 kg/hectare among smallholder farmers (Policy Analysis and Sustainable Agricultural Development in Central, Eastern Europe and Southern Africa (PASAD), 2005). For example, average prices of a 50 kg bag of fertilizers like 23:21:0 + 4S, UREA and Calcium Ammonium Nitrate (CAN) increased nearly fifteen times from an average of MK100.00[1] in 1994/95 to over MK1500 in 2004. In 2015, the average market price for UREA was MK 17,450. CAN was selling at MK17, 550 while 23:21:0 + 4S was selling at MK19, 600 per 50 kg bag. Taking into account the increasing costs of inorganic fertilizer, low-cost soil fertility improvements that enhance crop productivity like agroforestry need to be promoted.

The Malawi agricultural policy highlights the need for sustainable management and utilization of natural resources (Malawi Government, 2012). The National Land Resources Management Strategy calls for efficient, diversified and sustainable use of land based resources. Agroforestry is one of the potential interventions emphasized in the strategy (Malawi Government, 2000). Some of the agroforestry technologies being commonly practiced in Malawi include mixed intercropping, annual under sowing, dispersed systematic intercropping homestead, boundary and Woodlots (Malawi Government, 2012). In relay cropping, maize is planted at the onset of rain, but planting of the trees is delayed for about two weeks after the maize has been planted. Trees continue growing on the piece of land after the crop has been harvested, forming a short-term fallow during the dry season. Before the next rainy season, trees are cut and all the leafy biomass is incorporated into the soil and the poles are harvested for either light construction or fuel wood (Makumba, 2003).

Despite the emphasis of agroforestry in key national agricultural working documents like the agricultural policy, the National Land Resources Management Strategy, and Guide to Agricultural Production and Natural Resources Management Handbook, the agroforestry potential to improve maize production, scaling up remains the challenge in the

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[1] 1USD=MK710

country. The study was also commissioned on the background that the Malawi government through the department of Land Resources Conservation is currently developing a National Agroforestry Policy intended to scale up uptake of the technology by the small holders.

II. Purpose of the Study

The overall purpose of the study was to draw lessons for upscaling of agroforestry by identifying the factors that have led to no or low continuation of MAFE project activities after project phasing out in 2002.
Specifically, the study aimed to:

1. Determine factors affecting agroforestry technology scaling up;
2. Identify gaps in implementation and scaling up of agroforestry technologies; and
3. Recommend best-bet practices for scaling up agroforestry technologies.

III. Methodology

The study was conducted in Mzimba, Ntcheu and Mangochi districts in Malawi. In Mzimba district, the study was done in Kazombe Extension Planning Area (EPA); in Ntcheu district the study was done in Njolomole EPA, while in Mangochi district the study was done in Nthilamanja EPA. These EPAs were purposively selected for the study as they were part of the MAFE project sites and to have representation of all the three agro-ecological zones of Malawi (Low, Medium and high altitude). The study was undertaken in the 2011/12 cropping season.

Study population consisted of farmers who participated in the implementation of MAFE project activities (targeted) and those who did not participate (non-targeted) in the three EPAs. The entire populations of 164 farmers were interviewed in the study (83 targeted and 81 non-targeted). Simple random sampling was used to identify the farmers that participated in the study.

A Logistic regression model was used to examine factors responsible for scaling up of agroforestry technologies. The dependent variable was whether the farmer is still practicing agroforestry after MAFE project phased out in 2002. The model was chosen because the dependent variable had two possible outcomes 'Yes' and 'No' where 'Yes' = 1– farmer still practicing agroforestry after the project and 'No' = 0 – farmer not practicing agroforestry. As theoretically determined in literature, Logistic model was more appropriate for this analysis because it could identify the factors affecting scale up of agroforestry technologies (Agresti, 2007 and Gujarat, 2004). When the dependent variable is binary and can only take two values, use of ordinary multiple regression techniques and discriminant analysis are not suitable because a number of essential assumptions of such models are not satisfied and the predicted values cannot be interpreted as probabilities (Jabbar, Beyene, Saleeem, and Gebreselassie, 1998). Multinomial model would have been more applicable if there were three responses for the dependent variable: Linear regression was not used because it would violate the linearity assumption (Gujarati, 2004).

Logistic regression model also requires far fewer assumptions but directly estimates the probability of an event occurring or not occurring. In logistic regression, Forward Stepwise maximum likelihood method is used to estimate parameters (Jabbar *et al*., 1998). Statistical Package for Social Scientists (SPSS) was used for the data analysis. To determine the influence of perceptions (Perceived Ease of Use-PEOU and Perceived Usefulness-PU), age, education, income source, extension access, field day attendance, household land size, household size and household labour availability on agroforestry scale up, a regression using the binary Logistic model, using forward stepwise method was conducted (Field, 2000 and Gujarat, 2004). The dependent variable (Y) was a natural log of the probability of scaling up agroforestry technology or not.

The Logistic Model

$$\text{Logistic } [\text{ø}(x)] = \log [\text{ø}(x)/1-\text{ø}(x)] = \alpha + \beta_1 x_1 + \beta_2 x_2 + \ldots + \beta_i x_i + \ldots e \ldots\ldots \quad \text{Equation (1)}$$

Where:
α = the constant of the equation
hβ = the coefficient of the predictor variables.
E = error term
This can be simply expressed as follows

$$Y_1 = \ln (P/ 1-P) = f (X_1, X_2, X_3, X_4, X_5, X_6, X_7, X_8, X_9, X_{10}, X_{11}) + e \ldots\ldots\ldots\ldots \quad \text{Equation (2)}$$

Where:
Y_1 = whether the farmer adopted agroforestry technology after the project phased out or not
X_1 = sex of household head (male or female)
X_2 = marital status of the household head (single, married, widowed, divorced)

X_3 = age of the household head (number of years)
X_4 = education level of the household head (primary, secondary, tertiary, adult literacy)
X_5 = household main income source (selling own farm produce, small scale trading-owning a grocery, carpentry, buying and selling farm produce)
X_6 = extension access (visits to extension worker, visits by extension worker for advice, attendance of extension training and meetings)
X_7 = field day attendance (number of field days on soil fertility enhancing technologies attended)
X_8 = household land size (number of hectares)
X_9 = household size (total number of usual residents in the household)
X_{10} = household labour availability (conversion rates by availability of household member, sex and age)
X_{11} = Farmers Perceived Technology Characteristics (perceived usefulness (PU) and perceived ease of use (PEOU) using Technology Acceptance Model (TAM)
e = the error term

IV. Results and Discussion

a) Factors affecting scaling up of Agroforestry

The results showed that household main income source, extension access, household head education level, household number of fields, PU, PEOU and attendance to field days with emphasis on soil fertility enhancement technologies were significant at 5% (Table 1). The household's main source of income is the important factor to consider in scaling up agroforestry technology. According to Chamdimba (2003), agroforestry increases crop yield and consequently household income. This means that farmers whose main source of income is crop sales are more likely to invest in technologies that will increase their yield like agroforestry technology than those with other main sources of income. Hence working with those farmers is very crucial in scaling up agroforestry technologies.

This finding emphasizes the fact that the Department of Land Resources Conservation in the Ministry of Agriculture and Food Security in Malawi need to adopt a three-way strategy. Firstly, there is need to focus attention on low income and resource constrained farmers, especially poor resource farmers who cannot afford inorganic fertilizers. Secondly, there is need to promote co-management of soil fertility problem among the better-off households to obtain the best results from investment in mineral fertilizers because their households have access to inorganic fertilizer. Utilization of these organic technologies helps improve the organic matter content of the soil. Evidence shows that returns from use of inorganic fertilizers are maximized when organic and inorganic fertilizers are combined Thangata, P.H., Alavalapati, J.R.R. (2003). Lastly, farmers whose main source of income is crop sales should incorporate agroforestry trees, which can include homestead and field boundary planting in tobacco growing areas. This is because farmers whose main source of income is crop sale are likely to invest in technologies that will increase their yield output like agroforestry technologies. According to Kamoto *et al*., (2013) farmers are investing in tree management where benefits of investment are known. * < *0.05*

Table 1 : Factors for scaling up of groforestry

Characteristic	Coefficient	Std. Error
Sex	-0.106	0.446
Household size	0.006	0.033
Age of household head	-0.00	0.004
Main income source	0.109*	0.060
Education level	0.290*	0.166
Extension access	0.585*	0.233
Household land size (ha)	0.095	0.400
Field days	0.723*	0.302
No. of fields	-1.196*	0.564
Marital status	0.763	0.544
Labour	0.245	0.343
Perceived Usefulness	0.145*	0.086
Perceived Ease Of Use	-0.749*	0.223
Land ownership	-0.960	0.520
Club membership	0.267	0.476
Constant	01.035	0.986
R Squared	91.3	
Model Significant Level	0.000	
Number of observation	164	

Extension access and field days, which are the commonest ways of information dissemination channel, were also significant. This means that extension access by farmers, and participation in soil enhancement technologies, field days increases agroforestry technology scale up. It is inevitable that extension does create the necessary awareness to farmers and help to motivate more farmers to invest in agroforestry as an organic soil fertility technology. Educational level was also significant. Farmers' educational level is also important because literate farmers understand and practice modern farming technologies more than the illiterate ones and they can also be used as lead farmers. Matata (2009) reported that fellow farmers were 76% more effective in dissemination of improved fallow agroforestry technology information than government extension workers. This can be explained by the fact that farmers are able to understand each other better and appreciate what others are doing than just being told by government extension workers. The education level of the farmer is also expected to have a positive impact on the decision making process on agroforestry technologies scaling up. In a similar study by Ozor et al (2013), they found that farmers who were more educated were more willing to pay for improved agricultural technologies and extension services. It is expected that heads of households with six or more years of education will be able to understand the benefits of agroforestry. This is because at higher levels of education, the school curriculum may have covered general principles of agricultural and agroforestry practices. Educated farmers also read and write and have the ability to read '*Za a Chikumbi'*, a local farmers' newsletter produced by the Department of Agricultural Extension Services. The farmers who are able to read are more likely to be exposed to information regarding the environmental benefits of agroforestry (Thangata, 2003).

Perceived usefulness and perceived ease of use were also significant. These results call for intensive and proper sensitization and training of farmers on the benefit of agroforestry, so that when they clearly understand the usefulness of agroforestry, they cannot see it as difficult to practice and not useful. The perception that farmers have towards a technology plays a major role in influencing their beliefs about practicing that technology, beliefs influence their attitude about practicing/ using that technology, attitude influence their intentions to practice/use a technology and their intention determines the level practice/usage/adoption of the technology (Yang, 2004; and Burton-Jones and Hubona, 2006; Wang and Qualls, 2007). Age of the household head was not significant but it had negative coefficient.

b) Gaps in Implementation and Scaling up of Agroforestry Technologies

i. *During MAFE Project Implementation Period*

MAFE used sensitization meetings and community participatory appraisals to introduce the project to the communities. The project applied the principle of catchment conservation, where the whole community within the catchment area was involved in identifying the problems affecting their livelihoods and suggested possible solutions. In this process, EPA staff with help from district staff, chose the degraded catchments and sub-grouped the communities.

The MAFE project was imposed on farmers. They were just told that they will be involved in the project and that they will be given tree seeds and tree seedlings to plant in the fields, fields' boundary, homestead and woodlots. This shows that the farmers did not understand the project objectives well right from the beginning or that the sensitization was not very effective for farmers to conceptualize the project. It was found that this was so because sensitization meetings were done by government extension staff that had inadequate time, overloaded and preoccupied with other agricultural activities thereby not concentrating on MAFE project activities. Key informants recommended that for future projects the government should commit field staff on full time basis, unlike the setup of MAFE project where full time personnel were only at central level. It was also recommended that lead farmers should be elevated in order to fill the extension gap which may also affect future projects.

The study found that Farmers in the targeted sites were not fully sensitized because the project used top down approaches leading to the failure by most farmers to properly understand the objectives of the project. The study also found out that farmers were active during project implementation because they were given free tree seeds and seedling for their fields, woodlots and homesteads, which incentivized them. These were some of the reasons why most of the farmers did not continue with project activities after the project phased out in 2002. Similarly, the technologies did not scale up because of lack of incentives among non-targeted and other farmers after the project phase out.

The study also found out that the designing of the project was not flexible because it was not incorporating the changes during implementation based on lessons learnt. This means that future projects should use bottom up approaches, involve farmers during design and implementation, and be flexible to incorporate lessons learnt for effective project implementation and sustained outcomes. The project applied community based monitoring and evaluation approaches with farmers through field visits, review meetings and reporting. The project empowered EPA

staff to conduct the trainings while the project provided materials for the trainings. The trainings that were offered included, nursery establishment, tree nursery management and transplanting, field tree management, biomass incorporation, group dynamics, community based monitoring, problem diagnosis, seed collection and different types of agroforestry technologies. The study found out that although all the planned trainings were done in time in the initial years of the project, the trainings were done off season or not done at all in the later years. Where they were done, it only depended on the performance and commitment of the agricultural extension staff in the EPAs. This negatively affected the implementation of the project.

The project had limited coordination with other relevant stakeholders during project planning and implementation. The Land Resources Conservation Department, which coordinated implementation of the project, only worked in partnership with the department of forestry despite the existence of other relevant stakeholders like the Department of Environment, the Department of Energy and Natural resources, Forest Research Institute of Malawi and World Agroforestry Centre. There was no phase out strategy for the project resulting in an abrupt winding up of the project. This was also one of the reasons why the farmers could not sustain project activities and why the project did not scale up after phase out and that access to extension services greatly reduced after the project phased out.

ii. *Current Situation*

In Malawi, currently there is no specific strategy to scale up agroforestry in the country despite the presence of many players on the ground. This emphasizes on the need to finalize the development of Agroforestry Strategy of Malawi by National Agroforestry Steering Committee (NASC) of Malawi whose draft was produced in January 2008. The implementation of National Agroforestry Strategy will involve various stakeholders depending on their strengths. The National Agroforestry Research and Development Forum (NARDF) will have the overall responsibility and will be the custodian of the strategy. Finalizing the strategy will promote implementation and scale up of the agroforestry technologies and also coordination among different partners involved in agroforestry.

Currently there is high vacancy rate and farmer-extension worker ratio is out of proportion due to combined efforts of natural attrition and HIV/AIDS pandemic (1 extension worker to 2551 farm-families against the recommended ratio of 1: 1000 Malawi Government, 2012). The Department of Land Resources Conservation in the Ministry of Agriculture and Water Development in Malawi should identify and build capacity of more lead farmers on agroforestry technologies.

c) *Best- bet Technologies for Scaling up Agroforestry*

Best-bet Agroforestry technology is a technology which is culturally practiced and promising. It has proved popular, easy to manage, beneficial to farmers and fits well in the traditional cultures, value and farming practices. The study found different general best – bet technologies depending on the purpose of the agroforestry tree species (Table 2). Specifically farmers in Ntcheu, uses Tephrosia than other fertilizer agroforestry tree species due to its resistance to termites' attack and quick soil fertility restoration

Table 2 : Recommended best-bet technologies for scaling-up agroforestry

Agroforestry tree category	Agroforestry technology	Recommended Tree species
Soil Fertility Improvement	Undersowing, DSI	*Tephrosia vogelii, Tephrosia candida*and *Acacia polyacantha*
Fuelwood/Poles	Boundary, homesteads and woodlot planting	*Senna spectabilis, Senna siamea, Acacia galphinni* and *acacia polyacantha*
Fodder	Woodlots and field boundary	*Leucaena leucocephala*
Fruits	Homestead, field boundary and orchards	All kinds of fruit trees

V. Conclusion and Recommendations

The study concluded that scaling up of agroforestry technology can be achieved by providing quality and reliable agroforestry extension services and effective involvement of farmers and support of field staff in agroforestry projects is key for scaling up of the technologies.

The following recommendations were made

- Farmers and extension workers should be actively engaged in the design and implementation of agroforestry projects for scale up
- Agroforestry extension approaches like group mobilization, field days, follow up visits, demonstration plots and training should be promoted for farmers to perceive the usefulness of the technologies to enhance scale up.
- Future agroforestry projects should not provide free tree seeds and seedlings beyond initial year as they negatively affect project sustainability and scale up.
- Undersowing with *Tephrosia vogelii* should be promoted for quick soil fertility improvement and DSI with *Faidhabia albida* for its long term pattern

- The National Agroforestry Steering Committee of Malawi should finalize the development of agroforestry strategy and strengthen coordination and partnership among stakeholders involved in agroforestry.
- Homesteads and boundary planting should also be encouraged for soil fertility, food security, income, poles, and seed bank for technology scale up in tobacco growing districts. This so because farmers do not commonly plant agroforestry tress in tobacco fields.
- National tree planting season can be utilized to promote agroforestry tree planting,

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Bacteriological Quality Assessment of Raw Milk and Cheese in Selected Woreda of Wolaita Zone, Ethiopia

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Abstract- A cross sectional study was carried out at Wolaita Sodo district from May 2013 to November 2014 with the aim to evaluate the bacteriological quality and to isolate and identify common milk borne zoonotic bacterial pathogens (*Staphylococcus aureus, Escherichia coli,* and *Salmonella species)* from raw cow milk and cheese collected in market, restaurant and cafeteria. Milk quality related practice was also assessed by direct observation and questionnaire survey. A total of 56 raw milk and 40 cheese samples were collected in cafeterias / restaurants and from market. The result revealed that 43(76.7%) were poor quality milk with a total aerobic bacterial count more than $5x10^5$CFU/ml and only 13(23.3%) moderate or acceptable milk which have an estimated total aerobic bacterial count of below $5x10^5$CFU/ml. The mean total bacterial count and mean of coliforms count was significantly higher ($P<0.05$) in raw milk collected from market point than from cafeteria / restaurant.

Keywords: quality assessment, raw milk, cheese, coliform counts, total aerobic bacterial count.

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Bacteriological Quality Assessment of Raw Milk and Cheese in Selected Woreda of Wolaita Zone, Ethiopia

Ashenafi Kiros [α], Alemu Aylate [σ], Naod Thomas [ρ], Biniam Tadesse [ω] & Asefa Asmare [¥]

Abstract- A cross sectional study was carried out at Wolaita Sodo district from May 2013 to November 2014 with the aim to evaluate the bacteriological quality and to isolate and identify common milk borne zoonotic bacterial pathogens (*Staphylococcus aureus, Escherichia coli,* and *Salmonella species)* from raw cow milk and cheese collected in market, restaurant and cafeteria.Milk quality related practice was also assessed by direct observation and questionnaire survey. A total of 56 raw milk and 40 cheese samples were collected in cafeterias / restaurants and from market. The result revealed that 43(76.7%) were poor quality milk with a total aerobic bacterial count more than $5x10^5$CFU/ml and only 13(23.3%) moderate or acceptable milk which have an estimated total aerobic bacterial count of below $5x10^5$CFU/ml. The mean total bacterial count and mean of coliforms count was significantly higher ($P<0.05$) in raw milk collected from market point than from cafeteria / restaurant. The maximum and minimum total aerobic bacterial counts from study area were 8.00log10CFU/ml and 5.00 log_{10}CFU/ml, and coliform counts were 7.10 log_{10}CFU/ml and 4.23 log_{10}CFU/ml respectively. The mean coliforms and total aerobic bacterial count were significant ($P<0.05$) from different sampling point. The overall mean of total aerobic bacterial count and coliform count in cheese sample was 5.38log_{10}CFU/ml and 5.58 log_{10}CFU respectively. The bacteriological analysis revealed that 73.5% and 70% *Staphylococcus aureus* were isolated in raw milk and cheese respectively. A total 55.2% and 42.5% *Escherichia coli* and 42.9% and 30% *Salmonella species* were found in raw milk and cheese sample respectively.

Keywords: quality assessment, raw milk, cheese, coliform counts, total aerobic bacterial count.

I. Back Ground

The complex biological nature of milk makes it a suitable medium for growth of many microorganisms. During milk production it is impossible to avoid contamination of milk with micro-organisms; therefore, the microbial content of milk is a major feature in determining its quality (Rogelj, 2003). In order to make good dairy products, good quality raw materials are essential. A milk processor or handler will only be assured of the quality of raw milk if certain basic quality tests are carried out at various stages of transportation of milk from the producer to the processor (Haug, 2007).

Contamination of raw milk with bacterial and other microorganism can be from air, milking equipment, feed, soil, faeces and grass. The number and types of micro-organisms in milk immediately after milking are varied based on source of contamination. Poor feeding and housing strategies of cows may influence the microbial quality of milk. (Coorevits *et al*., 2008, Rogelj, 2003)..There is an increasing focus on milk quality and hygiene in the dairy industry. Producing high quality milk requires effective udder health programs at a herd level (Bhutto *et al.,* 2010).

Unlike in developed countries, the dairy industry in most African countries including Ethiopia is underdeveloped, dominated by unpasteurized milk and informal markets (Regional Dairy Trade Policy Paper, 2004).Wolaita Sodo and its suburbs is well known for their wealth in dairy products. In the study area still most of the market is informal and hence increased risk of microbial contamination. On the other hand, most of the milk consumed in rural areas is un-hygienically handled and preference is given to raw milk compared to pasteurized and boiled milk. However, no or limited studies on microbiological quality of raw milk and Cheese has done.

The study was conducted to assess the milk handling practices, to establish total plate count of bacteria and coliforms in raw cow milk and cheese from Wolaita Sodo, Areka, Humboand Boditi and determine the presence of selected milk-borne zoonotic pathogens such as *Staphylococcus aureus, Escherichia coli,* and *Salmonella* species.

II. Materials and Methods

a) Study area

This study was carried out from May 2013 to November 2014 in four woreda of Wolaita Sodo district, Southern Ethiopia. The area is located about 390 km south of Addis Ababa between 6°4´N to 7°1´N and 37°4´E to 38°2´E which is 700-2950 m above sea level. Wolaita zone has a total land area of 4537.5 square kilometers. The average annual rain fall of the study area is ranging from 450-1446 mm (WZFEDD, 2005).

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b) Study design and Sampling methodology

A cross sectional study was conducted from November 2013 to May 2014 to evaluate the bacteriological quality of raw cow milk and cheeses.

Based on the access of transport and vast milk production and marketing situations four woreda were purposively selected for this study. From three sampling points (cafeteria, restaurant and market) in all woreda's 14 raw milk samples and 10 raw cheese (Cottage cheese "ayib") were randomly collected. Aseptically collected milk and cheese samples for further bacteriological analysis were transported in ice box to veterinary microbiology laboratory which is found in Wolaita Sodo University.

Asemi structured questionnaire regarding general hygiene of handling milk and milk product transportation facilities and transportation instruments and others was prepared and 80 milk and cheese seller people was randomly subjected to different questions related to factors which reduces the milk and cheese quality. Some observations were made in all woredas up on sample collection.

c) Sample collection and transportation

A total of 56 raw milk and 40 cheese samples were collected from the study area to investigate the quality milk and cheese. All samples were collected aseptically and processed immediately through standard microbiological procedures as described in APHA (1992). A sample of 20 ml raw cow milk and 200g of raw cottage cheese "ayib"were collected from different sampling points such as restaurants, cafeterias and retail market in sterilized universal bottles. The samples were kept in icebox and transported to Veterinary microbiology laboratory within an hour's and then stored in refrigerator at 4°C. All samples were processed within 24hrs of sampling.

d) Bacteriological examination of collected samples

i. *Sample preparation*

Milk samples were homogenized in a blender at 600 rpm for 5-10 min and serially diluted up to 10^{-5} times by adding 1mL of the test portion into 9 mL of 0.1% sterile peptone water. The cheese samples intended for bacteriological analysis were ground into a fine paste using a sterile pestle and mortar before weighing. The total viable bacteria count was determined by adding 1 g of cheese sample into test tubes having 9 mL of sterile quarter strength peptone water. After thorough mixing using a vortex mixer, serial dilutions were prepared under safety cabinet and 1 mL of appropriate dilutions were pour plated in duplicate using standard plate count agar. The plated samples were allowed to solidify and then incubated at 32°C for 48 h.

ii. *Bacteriological analysis*

Standard laboratory examinations such as total aerobic bacterial count (TABC) and coliform count (CC) was undertaken to determination the bacterial load in raw milk and raw cheese samples. The total plate count agar (Hi media, India) was used for determination of total aerobic bacteria in milk n while the violet red bile agar (VRBA) (Oxoid, UK)was also used to determination of Coliform Count (CC).

Dilutions were selected so that the total numbers of colonies on a plate were not difficult to count. The media were prepared according to the guidelines given by the manufacturers. Total aerobic bacterial count (TABC) and coliform count made after plating and incubation of appropriate dilutions of milk and cheese samples in the standard plate count agar (SPCA) (Hi media, india) medium and in VRBA (Oxoid, UK)medium at 37°C for 48 hrs and 24 hrs respectively, following the standard procedures recommended by American Public Health Association (1992). After incubation, all colonies including those of pin point size in SPCA medium and purplish red colonies in VRBA medium were counted under colony counter and results from each SPCA plates which contained 25 to 250 colonies per plate whereas, less than 100 coliform colonies in VRBA were recorded. For colonies beyond this count the next dilutions were plated and similar procedure was followed.

After counting and recording bacterial colonies in each petridish the number of bacteria in milliliter milk was calculated by the following formula given by APHA (1992).

$$N = \frac{\sum C}{[(n1x2) + (0.1xn2)] \times d}$$

Where: N = number of colonies per milliliter of milk,
$\sum C$ = sum of colonies on plates counted,
n1= number of plates on lower dilution counted,
n2 = number of plates in next higher dilution counted and
d = dilution from which the first counts are obtained.

e) Identification of pathogens

A portion (1 g or 1 ml) from each sample was taken aseptically and inoculated in sterile Nutrient agar (Hi media, india) medium and incubated at 37 °C for 24 hours. Bacterial colonies grown on this medium undertaking for gram staining. Gram's positively reactive colonies were tested for catalase test and inoculated on sterile selective media (Mannitol Salt agar (M. S. A)) (Oxoid, UK) for *Staphylococcus aureus*, and grams negative bacterial colonies cultured in Eosin Methylene Blue (E. M. B) (Oxoid, UK)to investigate the presence of *Escherichia coli*, and Xylose lysine deoxycholate agar (X. L. D) (Oxoid, UK) and Rappaport-Vassiliadis brothmedium (Oxoid, UK) to identify *Salmonella* species.

The most common zoonotic milk borne pathogens was identified based on cultural

characteristics, gram nature and color of colonies. Identified colonies from each petriplate were picked, subcultured, incubated at 37 °C and then isolated by using various biochemical tests such as Catalase test, Coagulase, Indole test, Methyl red test, VogesProusker test, Nitrate Reduction test, Urease production, Citrate utilization test and Glucose, Lactose, Mannitol sugar fermentation test. These biochemical tests as stated below in table were performed to confirm, *Staphylococcus aureus, Escherichia coli and Salmonella* species.

Table 1 : Morphological & Cultural characteristics of Pathogenic bacteria isolated from Milk and Milk products

Sr. No	Isolated pathogens	Gramstaining and morphology	Culture characteristics on selective media
1	*S. aureus*	Gram + ve cocc	Mannitol Salt Agar (Golden yellow color colonies)
2	*E. coli*	Gram – ve coccobacilli	Eosin Methlene blue Agar (Green Metallic Sheen colonies)
3	*Salmonella spp*	Gram –ve rod shaped	Xylose lysine deoxycholate agar (Black Centre color colonies)

Table 2 : Biochemical characteristics of Pathogenic bacteria isolated from Milk and Milk products

Ser.N	Biochemical test	S. aureus	E. coli	Salmonella spp
1	Catalase	+	+	-
2	Urease	-	-	-
3	*Oxidase*	-	-	-
4	*Coagulase*	+	-	-
5	Citrateutilization	-	-	+
6	*Nitrate reduction*	+	-	-
7	*Indole production*	-	+	-
9	*Methylene red*	+	+	+
10	*Vogesproscuare*	+	-	-
11	Glucose	+	+	+
12	Lactose	+	+	-
13	Mannitol	+	+	+
14	*Sucrose*	+	+	-
15	*Maltose*	+	-	+

(Javed Khan et al, 2014)

f) Statistical Analysis

Data was evaluated using descriptive statistics and all counts were converted to logarism values to enable statistical analysis and to express count to $\log_{10}$ CFU/g, and to compare the results. The data was analyzed using SPSS version 20. One way ANOVA and one sampled T-test was performed to compare the mean of bacterial counts obtained from milk and milk product sample based on their location and to compare risk of higher source contamination respectively. Statistical significance is set at a P value of < 0.05.

III. Results

In this study a total 96 Milk and cheese samples were taken from Market, restaurant and cafteria from four selected woreda in the wolaita zone. Questionnaire survey on 80 milk seller from all woreda was made. The results of bacterial enumeration, isolation in milk and cheese and associated risk factors are shown in as follows.

In the present study, roughly all milk sellers were female farmers and more than half of them brought their milk in open pots, jericans, cheap recycled dirty plastic containers and their cheese in local inset leaf and sell it directly in the market without any processing and packaging. From a total of 80 peoples subjected for the questionnaire, only 15 (18.7%) of them camefrom the city and 65 (81.3%) were from nearby villages.

Based on the questionnaire survey 86 % of the respondents were lack of awareness on hygienic handling of milk and cheese, deficient in clean water, used poor type of barn, poor hygiene milking. Nearly all milk sellers were complained for lack of transport facilities rather transported on rough roads over long distances without cooling and under high ambient temperatures and sell their milk and cheese under dusty and hot conditions. Very insignificant numbers of the respondents were hygienically practiced in their farms.

a) *Total bacterial and coliform count in raw milk and cottage cheese*

The bacteriological analysis revealed that 43(76.7%) from the total of 56 raw milk samples, were found to be poor quality milk with a total aerobic bacterial count more than $5x10^5$CFU/ml. However, moderate or acceptable quality milk samples accounts only 13(23.3%) which have an estimated total aerobic bacterial count of below $5x10^5$CFU/ml as shown in table 1.

Table 3 : The comparison between means of total bacterial (TABC) count in raw milk and raw cheese

Type sample	N	Means	t -value	dfp	- value
Cheese from market	20	5.58	-0.443	19	0.33
Cheese from cafeteria/ restaurant	20	5.52			
Total	40				
Raw milk from market	28	6.37	2.438	27	0.011
Raw milk from cafeteria/ restaurant	28	6.08			
Total	56				

b) *Total bacterial and coliform count in raw milk and cheese*

Based on the present study the mean total aerobic bacterial count of were 6.22$\log_{10}$CFU/ml and 5.38 $\log_{10}$ CFU/ml raw milk and cheese respectively. The maximum and minimum total aerobic bacterial counts from study area were 8.00Log10 CFU/m and 5.00 in raw milk and $\log_{10}$CFU/ml 6.10Log10CFU/ml and 4.10 $\log_{10}$ CFU/ml in cheese.

The overall mean of coliform count were 5.6 $\log_{10}$CFU/ml and 5.58 $\log_{10}$ CFU in raw milk and cheese respectively. The maximum and minimum coliform count in raw milk and cheese were 7.10 $\log_{10}$CFU/ml and 4.23 $\log_{10}$CFU/ml, and 7.10 $\log_{10}$ CFU/ml and 4.23 $\log_{10}$ CFU/ml respectively. The mean of coliforms and total bacterial count were significantly ($P<0.05$) different in the selected woredas. Humbo was identified with peak mean of total bacterial and coliform count in raw milk among the study areas Table 3

There was also significant difference ($p<0.05$) between counts of total bacteria and coliformis in cheeses collected from different sampling areas. The highest total bacterial and coliform count was recorded in Humboworeda.

Table 4 : Overall mean (±SE) of total bacterial counts (TABC) ($\log_{10}$CFU/ml milk)

Study area	sample type	N	Means ±SE	Minimum	Maximum	95 % CI Lower	Upper
Boditi	Milk	14	6.54 ±.483	6.17	7.30	6.26	6.82
	Cheese	10	5.52 ±.687	4.10	6.07	5.02	6.01
Humbo	Milk	14	7.02 ± .603	6.20	8.00	6.67	7.37
	Cheese	10	5.84± .406	5.05	6.10	5.55	6.13
Sodo	Milk	14	5.70 + .497	5.00	6.20	5.41	5.99
	Cheese	10	5.29±.465	4.81	6.04	4.96	5.63
Areka	Milk	14	5.61 ± .366	5.14	6.38	5.39	5.82
	Cheese						
Total	Milk	56	6.22±.764	5.00	8.00	6.01	6.42
	Cheese	40	5.38±.660	4.10	6.10	5.17	5.59

Milk- p- value-0.001 df =3 and cheese- p- value-0.014 df =36

Table 5 : Overall mean variation of coliform count ($\log_{10}$CFU/ml milk) and cheese

Study area	sample type	N	Means ±SE	Minimum	Maximum	95 % CI L ower	Upper
Boditi	Milk	14	6.12 ± .086	6.00	6.26	6.07	6.17
	Cheese	10	5.72±.723	4.10	6.41	5.20	6.23
Humbo	Milk	14	6.24 ± .354	5.90	7.10	6.03	6.44
	Cheese	10	5.94 ±.319	5.05	6.11	5.71	6.17
Sodo	Milk	14	5.19 ± .503	4.23	6.10	4.89	5.47
	Cheese	10	5.29 ±.465	4.81	5.04	4.96	5.63
Areka	Milk	14	5.46 ± .472	5.00	6.14	5.20	5.74
	Cheese	10	5.37 ± .551	3.70	6.11	4.97	5.76

Total	Milk	56	5.76± .583	4.23	7.10	5.99	5.91
	Cheese	40	5.58 ± .578	3.70	5.04	5.3970	5.76

Milk- P value- 0.000 df =3 and cheese- p- value-0.035 df =3

c) *Identification of the isolates*

Table 5 : Distributionmilk borne zoonotic bacterial pathogens in raw milk and cheese

Bacteria isolates	Types of Sample	
	N (56raw milk)	N (40 raw cheese)
Staphylococcus aureus	41(73.5%)	28(70%)
Escherichia coli	31 (55.2%)	17(42.5%)
Salmonella species	24 (42.9%)	12(30%)

The study revealed that *Staphylococcus aureus* were dominantly contaminating the raw milk and cheese samples 73.5% and 70% respectively. Of the total samples 73.5% and 70%, 42.9%, 30% raw milk and cheese respectively, were contaminated with *Escherichia coli* and *Salmonella* species correspondingly.

IV. Discussion

According to the respondents the major milk quality related constraints was lack of awareness on hygienic handling , being deficient in of clean water, poor type of barn, poor Hygiene of the milker, lack of transport facilities, and inappropriate materials used for milk production and handling. Milk and cheese for market in these study areas are also transported on rough roads over long distances, in cheap recycled dirty plastic containers without cooling and under high ambient temperatures and sells their milk and cheese under dusty and hot conditions. This is in agreement with (Parekh & Subhash, 2008) reason that use of unclean milking and transport equipments contribute to the poor hygienic quality of milk and cheese. Another possible source of contamination microorganisms in the study area was unclean teats, poor health of dairy herd and unhygienic milking conditions. It is clear that many zoonotic diseases are transmissible via milk and milk products. The strong traditionally habit of the people in the study area for utilizing raw milk and milk products were greatly at risk of obtaining these pathogen.

One sample t _ test was used to compare the total viable bacterial and coliform count in raw milk and raw cheese from open market and from cafeterias / restaurants to determine range of contamination. The mean total viable bacterial in raw milk from cafeteria and restaurants were significantly lower than in open market but, is slightly higher in raw cheese from open market than cheese from cafeteria and restaurant. This result was in line with reports by Ashenafi (2006), Biniam, (2006), Zelealem et al (2007b) and Seifuetal (2013). The mean count of coliforms was notably higher in raw milk and raw cheese collected from market than from restaurants and cafeterias. Higher bacterial count in raw milk and cottage cheese from open market is because of immense source contamination from different hands in the market, dusty and hot selling conditions, utensils used for transportation. The less bacterial count in the samples collected from the cafeteria and restaurants was possibly because of optimum storage and not open unlikely to that of retail market selling environment. More or less all cafeteria and restaurants had a refrigerator and bought their milk from small scale farms in the town not necessarily from open markets.

The total bacterial load obtained from raw milk sample in this study was high (6.22 $\log_{10}$CFU/ml) as compared to the acceptable value. This finding is in line with the finding Monika. S *et al,* (2013) 6.35 log10CFU/ml, but higher than Karmen *et al*. (2008) 4.5 log10 cfu/ml. It was appreciably less than result reported by Simenew *et al*. (2013), 7.34 in private dairy commercial farm but slightly lower by the same author (6.46) in government dairy farms.

V. Conclusion and Recommendations

Based on the current findings of this study, it is concluded that:

Milk produced by farmers and supplied to market in Wolaita sodo districts contains unacceptable levels of hygiene indicators and indicates a potential source of milk-borne infections. This raises a public health concern about its safety to consumers. Since raw milk is an important vehicle for transmission of zoonoses and other pathogens, this microbial status implies that milk consumers in the study area are at health risk. Indeed, this is supported by detection of Staphylococcus aureus at higher prevalence.

It is therefore recommended that:

1. Veterinary/extension animal health services should be provided to livestock farmers on proper animal husbandry and control of diseases.
2. It is recommended that routine assessment of milk quality produced and consumed by the public be mandatory in order to safeguard the public from milk-borne zoonotic diseases which may emanate through consumption of unsafe milk and milk products.

3. There should be implementation of good hygiene practices throughout the milk chain by training of all stakeholders involved in milking, milk collection and processing, including pasteurization, transport, and delivery, to ensure the safety and quality of milk.
4. Consumer practices, such as milk boiling, to reduce or eliminate potential infection by milk-borne zoonoses should be further encouraged.

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Conflict of Interest Statement

Authors have declared that no competing interests exist.

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Sweet Sorghum: A Sweet Grass for Bioenergy

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Abstract- *Sorghum bicolor* (L.Moench) is one of the most important multipurpose crop for production of golden syrup and treacle and alcohol from stalk juice. Its bagasse and green foliage could be used as an excellent fodder for animals, as organic fertilizer or for paper manufacturing. Sweet sorghum is a high-biomass and sugar yielding C4 plant containing approximately equal quantities of soluble glucose and sucrose, and insoluble carbohydrates (cellulose and hemicelluloses). Sorghum has been shown to be excellent silage in many areas of the world. Plant cell walls are vast reserves of photo synthetically fixed carbon. The brown midrib mutants have been used to identify and characterize the genes that encode the major enzymes for specific steps of monolignol biosynthesis for sorghum.

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I. Introduction

Sorghum bicolor L. Moench is commonly called as sorghum and also known as durra, jowar or milo belongs to family graminae. It is a genus with many species and subspecies and there are many types of sorghum, which includes grain sorghum, sweet sorghum and broomcorn.

Sorghum ranks fifth in worldwide among cereal crops. In India sorghum is grown for multiple purposes besides grain, the stem is used as fodder, fuel and fencing material. In many parts of Asia and Africa, their grains are used to make breads that form the staple food of many cultures. The grains can also be popped in a similar fashion to popcorn. Sorghum[*Sorghum bicolor* (L.) Moench.] is an important forage crop for grazing dairy cows in many milk producer regions of the world as frequent drought and high summer temperatures reduce forage production from pastures. Forage sorghums can be planted later than corn; use water much more efficiently; and, when exposed to drought, still produce acceptable yields [15].

Sorghum is C4 crop and tolerant to various abiotic stresses *viz.,* salt, water and drought. Therefore, it can be grown in arid regions. Bio-fuels (bio-ethanol and bio-diesel) produced from renewable energy sources are gaining importance in the light of rising fossil fuel prices, depleting oils reserves and increasing 'greenhouse emissions associated with the use of fossil fuels. Several developing and developed countries have made a mix of policies to promote the production and use of bio-fuels. Ethanol accounts for 90% of total bio-fuels production and use in different parts of the world at present. As bio-fuels are produced from biomass of crop plants, they offer enormous opportunities to improve the income levels of smallholder farmers in developing countries. At community level, farmers can cultivate energy crops that fetch more income while meeting their food needs. Local production of bio-fuels is projected to have a broad range of positive economic, social and environmental implications. Biomass is the oldest source of energy and currently accounts about 10% of total primary energy consumption. Traditional biomass in the form of wood fuel is still the main source of bioenergy.

Bioethanol production has shown rapid growth during last decade. In 2008, global biofuel production reached about 83 billion liters, a more than fourfold increase compared to 2000 production volumes. The United States and European Union are amongst the largest producers of biofuel, emerging and developing countries increased their share to about 1.5% of global transport fuel consumption. This demand of fuel will be increasing in coming decades. In most developing countries clean cooking fuels is more urgent than the supply of clean transport fuels. Using lignocellulosic–biomass as feedstock, second- generation biofuels could avoid competition with food production and at the same time increase income opportunities for rural farmers. Cellulosic ethanol is a biofuel produced from wood, grasses, or the non-edible parts of plants .It is a type of biofuel produced from lignocelluloses, a structural material which makes most of the biomass of plant[4,18] reported several types of lignocellulosic biomasses have been explored as a feedstock for the production of bioethanol which include agriculture residue, soft and hard wood, waste paper and energy crops. Lignocellulosic biomass consists ofpolymers such as cellulose, hemicellulose and lignin[18,21]. These polymers are arranged in such a way that they make the biomass complex and recalcitrant. Lignocellulose is composed mainly of cellulose, hemicellulose and lignin. Sorghum, Corn, Switch grass and Miscanthus are popular cellulosic material for ethanol production. There are three ways of producing ethanol from cellulose.

Cellulolysis processes which consist of hydrolysis on pretreated lignocellulosic material, using enzymes to break cellulose into sugars such as glucose followed by fermentation and distillation. Gasification is the process that transforms the lignocellulosic raw material into gaseous carbon monoxide and hydrogen. These gases can be converted into ethanol by fermentation.

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a) Sorghum as feedstock

There is a growing interest for alternative energy sources because of the fossil fuel crises. Ethanol used as automotive fuel has increased at least six times in the current century. According to the Renewable Fuels Association, in 2010 the USA bio-refineries generated 13 billion gallons of bio-ethanol and the year before worldwide production reached 19 billion. This noteworthy increment is in its majority based on maize and sugar cane as raw materials (Berg, 2004; Renewable Fuels Association, 2010)

IEA (2010) provides a clear definition of first generation biofuels and second generation biofuels. Sorghum is a multipurpose crop with the potential to achieve sustainable biofuel production, human food and animal feed products. Typical first generation biofuels are sugarcane ethanol, starch-based or "corn" ethanol and biodiesel. Sugar rich crops especially those that yield multiple end products, are promising. Alternative energy sources that are cost effective and technologically sound need to be developed. First generation biofuels, like ethanol and fatty acids ethyl esters mixed with gasoline and diesel are used in several countries.

b) Ethanol production

Sweet sorghum is an attractive feed stock for ethanol production. The juice extracted from the stalk contain soluble sugars and can be fermented into ethanol [21].Sweet sorghum is sugar crop with biofuel potential and found to be competitive with corn for theoretical ethanol yield with less input of energy.(Smith etal., 1987; Smith and Buxton, 1993; [8].If starch and cellulosic ethanol are considered sweet sorghum likely produce 50%-100% more ethanol per acre than corn grain and stover[14]. In sweet sorghum the sugar is contained in the main stalk, and is recovered by pressing the stalks with rollers (similar to the process used for sugar cane). Yields, on average, are 20 gallons of ethanol per ton of stalks [7].The average ethanol production for the Top 76-6 variety was approximately 220 g ethanol per kg of dry stem, which is equivalent to 2465 l of ethanol per ha.[9].Sweet sorghum, also known as sugar sorghum, belongs to the common grain sorghum plant. It is regarded as the most promising feedstock source for ethanol production because of several advantages, including rich germplasm resource, high biomass yield, rapid growth, wide adaptability, rich sugar content in stalk, clean and relatively low production cost [6,23,1,10,11,12,13,25].

Production of bioethanol from lignocellulosic biomass through a biological route involves three major steps: pretreatment, enzymatic hydrolysis, and fermentation [3]. Pretreatment is a critical step. The purpose of pretreatment is to break up the lignin seal, prehydrolyze the hemicellulose, and disrupt the crystalline structure of the cellulose, thus allowing cellulases better access to cellulose during enzymatic hydrolysis [5,9,22,4]. The bioconversion efficiency of *N. crassa* DSM 1129 was found superior to *S. cerevisiae* 2541. *N. crassa* fermented both glucose and pentose sugars present in SB cellulose and hemicellulose, reaching almost double ethanol production than *S. cerevisiae*.

c) Biomass

Lignocellulosic materials are the most promising feedstock as natural and renewable resource essential to the functioning of industrial purposes. A considerable amount of such materials as waste byproductsare being generated through agricultural practices mainly from various agro based industries. [16]

Biomass energy has the potential to greatly reduceour greenhouse gas emissions. Biomass creates about the same amount of carbon dioxide as fossil fuels, but every time a new plant grows, carbon dioxide is actually removed from the atmosphere. The net emission of CO_2 will be zero as long as plants continue to be replenished for biomass energy purposes. These energy crops, such as fast-growing trees and grasses, are called biomass feed-stocks. The use of biomass feed-stocks can also help increase profits for the agricultural industry. Agro-industrial biomass comprised on lignocellulosic waste is an inexpensive, renewable, abundant and provides a unique natural resource for large-scale and cost-effective bio-energy collection [26]Dedicated biomass crops are typically non-food crops grown as feed-stocks for the purpose of transportation fuel, energy production and a wide range of industrial end uses. Instead of crop residue, dedicated biomass crops such as Sorghum Miscanthus and switchgrass among others, could be used for bioenergy production since both species have been reported to produce high yields of biomass with relatively low nutrient. Plant biomass is the most important trait because of abundant amount of Cellulose which can be converted into monomeric sugars.

d) Brown midrib sorghum

Wild relatives of sorghum species and land races are good source of genetic variation. Brown midrib sorghum Sudan grass could be exploited to create varieties with low lignin and high biomass through introgression breeding. Forage sorghum developed and bred to contain the BMR gene, has less lignin and will be good substrate for cellulolytic enzymes. Less lignin ensures the plants are softer and easier to apply pretreatment methods.

A class of low lignin mutants that were discovered in maize (first identified in Minnesota) (Jorgenson L.R 1931) were the brown midrib mutants(bm)The bm mutants were named based on the red-brown coloration of lamina midrib, which intriguingly accompanied low levels of lignifications in stem

tissue(20 Nelson OE 1964,. General screening of chemical mutagenesis populations subsequently expanded the number of allelic and non-allelic brown midrib lines in maize and sorghum [2].

The brown midrib trait was discovered during the 1930's at Purdue University and early breeding work identified reduced vigor and yield concerns. These problems have been overcome and results show reduced lignifications, reduced cell-wall concentration, and increased palatability. Of 38 bmr mutant reported in sorghum four have been identified as individual group bmr2, bmr6, bmr12, bmr19 based on allelic test. Brown midrib (*bmr*), tan coloration to reddish brown of leaf midrib is a morphological marker to identify a popular genetic mutation in C4 grasses. Brown midrib, a genetic mutation in several grassy species, reduces lignin content in the total plant parts. Lignin is mostly indigestible, supporting material and in plants. During the past several years the brown midrib (*bmr*) trait has been introgressed into forage sorghum, Sudan grass, and corn. The results have been significant for the most part. IVTD values for *bmr* sorghum have demonstrated that differences between corn and sorghum silages have been removed.

Brown midrib (*bmr*) mutations in sorghum is characterized morphologically by brown vascular tissue in leaf blade and sheath, as well as in stem [17].Low lignin bmr-mutant of forage sorghum stalk, lignin content of sorghum bagasse, wheat straw, miscanthus and switch grass were evaluated and compared for bio-ethanol production. Also enzymatic hydrolysis yield of sugars and fermentation yield of ethanol studied.[24]

List of *bmr* source and derivative evaluated for biofuel traits

Sr No	Entries	Traits	BMR source/Derivative
1	IS 23253	New bmr source	Bmr source
2	IS 23789	Bmr 6	Bmr source
3	IS 21890	Bmr7	Bmr source
4	IS 23787	Bmr6	Bmr source
5	IS 21887		Bmr source
6	SPV 2017	Tall, bmr	Bmr source
7	SPV 2018	Tall,bmr	Bmr source
8	COS 26 X IS 21888	Mid tall	Derivative
9	PC 5 X IS 21888	Mid tall	Derivative
10	Palem 2 X IS 21891-2	Mid tall	Derivative
11	EC 582508 X NSSV 352	Mid tall	Derivative
12	BN 111 X(CSV 15X IS 21891)	Tall, high biomass	Derivative
13	SSV 84 X IS 21890	Mid tall, broad leaves	Derivative
14	RS 647 X EC 582508	Mid tall, broad leaves	Derivative
15	NSSV 258 X EC 582508-1	Mid tall, broad leaves	Derivative
16	NSSV 258 X EC 582508-2	Mid tall, broad leaves	Derivative
17	[(CSV 15 X IS 21891-1-1-1)X(HC 260XB 35)-2-1-1-1	Mid tall, broad leaves	Derivative
18	EC 582508 X RS 647	bloomless	Derivative

e) Current Status

At present, researchers are more interested in sugars-based ethanol (first generation ethanol)which can also be used as food source (examples are corn, sorghum and sugarcane) to non food based ethanol (second generation ethanol) such as lignocellulosic biomass and as a consequence limit the competition between fuels and food production. Field crops are one of the best sources of renewable energy which can be used as feed-stock for biofuels production. The cell wall of plant biomass consists of several major polymers: cellulose, hemicellulose, lignin, and minor components such as organic acids, proteins, tannins as well as secondary metabolites of intrinsic value. A complete understanding of the lignin biosynthetic pathway, including the underlying regulatory mechanisms, is essential for efficiently engineering and harnessing the energy stored in lignocellulosic material.

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Prevalence and Public Health Importance of *Cystcercus.Bovis* from Cattle Slaughtered in Mekelle Municipal Abattoir, Tigray, Ethiopia

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Abstract- A cross sectional study was conducted from November 2015 to March 2016 to estimate the prevalence of Cysticercosis, to investigate prevalence and rate C.bovis infection with in different risk factors and to determine the distribution and viability of cysts in organs of animals slaughtered in Mekelle municipal abattoir. Ante-mortem and postmortem examination based on the standard and routine procedure were performed on randomly selected apparently healthy cattle brought for slaughter during period of the study. Of the total 312 inspected animals, 21 animals were found infected with C. bovis distributed in different anatomical structures with an overall prevalence of 6.7% (21/312). Even though there is no statistical significant difference among different ages groups, sex, breed and body condition score of the animals, the prevalence was roughly varied.

Keywords: *abattoir, cattle, c.bovis, cross sectional, mekelle, prevalence.*

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Abstract- A cross sectional study was conducted from November 2015 to March 2016 to estimate the prevalence of Cysticercosis, to investigate prevalence and rate *C.bovis* infection with in different risk factors and to determine the distribution and viability of cysts in organs of animals slaughtered in Mekelle municipal abattoir. Ante-mortem and postmortem examination based on the standard and routine procedure were performed on randomly selected apparently healthy cattle brought for slaughter during period of the study. Of the total 312 inspected animals, 21 animals were found infected with *C. bovis* distributed in different anatomical structures with an overall prevalence of 6.7% (21/312). Even though there is no statistical significant difference among different ages groups, sex, breed and body condition score of the animals, the prevalence was roughly varied. Relatively higher prevalence of infection was found in adult 15(71.4%) than that of old 6 (28.6%). Generally prevalence of C*ysticercos is bovis* was prominent in males 20(95.2) unlike female animals 1(4.8). About 269(86.2 %) examined animals were local breed with considerably higher prevalence 20(95.2%). Based on body condition score, reasonably greater prevalence was found in good body condition animals 10(47.6) followed by 8(38.1) and 3(14.3) in medium and poor conditioned animals, respectively. Noticeably higher number 234(75%) of cattle slaughtered in Mekelle abattoir came from highland area with fairly increased prevalence 15(71.4%). Moderately higher rate of infection was recorded within old cattle 8.8% (6/68) compare to adult animals 6.15 %(15/244). Regarding cyst locations, out of the total 21 cattle positive for *C. bovis*, carcasses of 12 cattle were having the cysts at one site only and 9 at more than one site. More cysts were encountered in tongue. From a total of 21 identified cyst positive carcasess 6 were containeda viable cyst. The overall findings of this study indicated the importance of *C. bovis* should not be under estimated.

Keywords: abattoir, cattle, c.bovis, cross sectional, mekelle, prevalence.

I. Introduction

The metacestodes (or larval cestodes) of Taenia spp. tapeworms are the cause of cysticercosis in various farmed and wild animals and in humans. Adult tapeworms are found in the small intestine of carnivore definitive hosts: humans, dogs, and wild canids. Taeniasaginata of humans causes bovine cysticercosis, which occurs virtually world-wide, but particularly in Africa, Latin America, Caucasian and South/Central Asia and eastern Mediterranean countries. The infection occurs in many countries in Europe and sporadically in the United States of America (USA), Canada, Australia and New Zealand (OIE, 2014).

Recently, the World Health Organization (WHO) included cysticercosis as part of the Neglected Zoonosis subgroup for its 2008–2015 strategic plans for the control of neglected tropical diseases WHO (2013). This disease causes considerable livestock-associated financial losses and represents a significant food safety problem. The epidemiology of bovine cysticercosis/human taeniasis various from one area to another so control measures appropriate in one area is not necessarily of value in another. Based on routine carcass inspection, the infection rate of bovine cysticercosis is often around 30-60% in developing countries although the real prevalence could be considerably high (Tembo, 2001). In Ethiopia, there are different reports regarding the prevalence of taeniasis. The prevalence of cysticercosis reported was between 3.1 and 4.9% in Central Ethiopia, Jimma and Gondar (Tembo, 2001; Dawit, 2004; Megersa *et al.*, 2009; Taresa*et al*. 2011), and 26.3% in Hawassa and Wolita Sodo (Abunna*et al*., 2008; Regassa*et al*., 2008), based on Abattoir survey.Hence, it is essential to have adequate knowledge of the epidemiology of the disease before contemplating control programmes. Moreover, estimation of the cyst viability is important for decision making, planning of development and implementation of control strategies. Despite the presence of high prevalence of the disease in other areas only a limited studies has so far done in the current study area. Hence, no adequate information is available regarding epidemiology and public health significance of the diseases.

Therefore, this study was conducted with the following objectives;

- To study the prevalence of cysticercosis in animals slaughtered in the abattoir

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- To investigate prevalence and rate *C.bovis* infection with in different risk factors
- To determine the distribution and viability of cysts in different organs

II. Materials and Methods

a) Study Area

The present study was conducted from November, 2015 to March, 2016 at Mekelle Municipality Abattoir. Mekelle is the capital city of Tigray Regional State and is located 783kms North of Addis Ababa. It is located between altitudes of 2000-2200 m above sea level and has moderate (Woynadega) zone of climatic conditions. Geographically, Mekelle city is found in 390 28´ east and 130 32´ north. The average annual rainfall of the city ranges from 50-250 mm and the average mean temperature is 19^{0}CTesfu Weldegerima (BOFED), 2008.

b) Study Design

A cross-sectional study was conducted on prevalence of *C. bovis* from November, 2015 to March, 2016 at Mekelle Municipality Abattoir.

c) Study Population

Study population was cattle presented to Mekelle municipal abattoir for slaughtering. The study population consists of 312 cattle at different ages, sex, origins, body condition and breeds categories in the study area. The sources of animals for the study were from high land and low land areas. The slaughtered animals in the abattoir were inclusive of the two sexes (males and females).

d) Sample Size Determination

The desired sample size was calculated using the formula recommended by Thrusfield (2005) with 95% confidence level, 5% desired absolute precision and expected prevalence.

$$N = \frac{1.96^2 P_{exp} (1-P_{exp})}{d^2}$$

d=absolute precision (usually 0.05)
Where, n=required sample size
P_{exp} =expected prevalence

Previous study made in Mekelle abattoir showed the prevalence of *C. bovis* in cattle to be 7.23% (Getachew and ashewani, 2008). Therefore, by using this 7.23% expected prevalence, at a confidence interval of 95% and required absolute precision of 5%, accordingly, a total of 104 cattle were supposed to be sampled. However, to raise the level of accuracy of prevalence determination, the sample size was increased to 312 in the current study.

e) Sampling Technique, Sample Collection, and laboratory Procedure

According to the guideline by Ministry of Agriculture (1972), routine meat inspection technique was employed for detecting *C. bovis* cysts, the meat was inspected visually, followed by a longitudinal ventral incision of the tongue from the tip of the root, one deep incision into the triceps muscles of both sides of the shoulder, deep incision into external and internal muscles of the masseter parallel to the plane of the jaw, longitudinal incision of the heart from base to apex, 3 parallel incisions into the long axes of the neck muscles on both sides as well as one extensive incisions on the diaphragm; visual examination, palpation and incision of lung, liver and kidneys. Findings were registered according to the organs inspected.

From cyst positive organs during meat inspection cysts and surrounding tissues were trimmed and transported in cold chain to parasitology laboratory in Mekelle University, College of Veterinary Medicine for viability tests.

Cyst viability test

The viability of the cyst was examined by using ox bile solution diluted in normal saline and incubated at 37oc for 1 to 2 h. A cyst was regarded as viable if the scolexevaginated according to (Graceyet *al*., 2009).

f) Data Management and Analysis

The data was checked, coded and entered in to Microsoft excel work sheet and analyzed using SPSS software. Descriptive statistics like percentage was used to express prevalence, while chi-square ($\chi 2$) test was used to compare the association of cysticercosis with different risk factors. In all the cases, 95% confidence level and 0.05 absolute precision errors were considered. A p-value $<$ 0.05 was used to verify statistically significance difference.

III. Results

The presence of *Cysticercus bovis* in various organs of 312 cross and local breed cattle was investigated. Out of 312 cattle inspected, only 21 animals were found positive with an overall prevalence of 6.7%.

a) Computing Different Risk Factor with C.bovis Prevalence

A total of312 slaughtered cattle in Mekelle municipal was computed for the prevalence of *Cysticercus bovis* based on their ages, breed, body condition score, origin, and sex (table 1). Even though there was no statistical significant difference among different risk factors, the prevalence was roughly varied. Relatively higher prevalence of infection was found in adult 15(71.4%) than that of old 6 (28.6%). Generally prevalence of *cysticercus bovis* was prominent in female

animals 20 (95.2) unlike males 1 (4.8%). About 269(86.2 %) examined animals were local breed with considerably higher prevalence 20(95.2%). Based on body condition score, reasonably greater prevalence was found in good body condition animals 10(47.6) followed by 8(38.1) and 3(14.3) in medium and poor conditioned animals, respectively.

Table 1 : Association of *C.bovis* prevalence with different risk factors

Variables	Categories	Total examined animals (N)	No. of infected animals (%)	X^2	*P-value*
Age	Adult	244	15(71.4)	0.607	0.436
	Old	68	6 (28.6)		
Origin	Low land	78	6(28.6)		
	High land	234	15(71.4)	0.153	0.696
Sex	Male	298	20 (95.2)	0.004	0.951
	Female	14	1 (4.8)		
BCS	Poor	37	3(14.3)	1.664	0.435
	Medium	161	8(38.1)		
	Good	114	10 (47.6)		
Breed	Local	269	20(95.2)	1.542	0.214
	Cross	43	1(4.8)		
Total		312	21(6.7%)		

b) Rate of Infection with in Different Risk Factors

Rate of infection within adult animals was 6.15 %(15/244). However, within old cattle the rate of infection was 8.8% (6/68). Male animals were significantly found positive for cysticercus bovis with infection rate of 6.7% (20/298) than females 7.1% (1/14). Among local animals 20 were found infected. Rate of infection within local breed animals was 7.4 %(20/269). On the other hand rate of infection in cross breed cattle was 2.3 % (1/43). The body condition of animals and the presence of infection were also investigated. Along with good conditioned animals, 10 were found infected with infection rate of 8.8% (10/114). Whereas, (8/161) 5% and (3/37) 8.1% infection rate was identified with in medium and poor body condition scored cattle respectively. The rate of infection in high land was 6.4% (15/234). On the other hand there was 7.7 %(6/78) infection rate in low land cattle.

c) Distribution of Cyst in Different Organs and its Viability

Regarding cyst locations, a total of 21 cattle were found positive for the presence of C. bovis on their different organs (table 2). Carcasses of 12 cattle were having the cysts at one site only and 9 at more than one site. There was variation in the number of cysts in different organs. Large number of cysts (14) was found in tongue of examined animals. The other organs of cattle carcass found positive for C. bovis were liver, 8, masseter muscles, 4, heart, 4, triceps muscles, 2 and lung 1. Cysts from positive organs were subjected for viability test. Relatively not few cysts were contained viable infection. Six cysts out of the total 21 tested cysts were having viable infection.

Table 2 : Distribution of cyst in different organs and its viability in percent

Organs inspected	No cyst distribution	No. of viable cysts (%)
Tongue	5	0
Liver	5	1(4.76)
Heart	1	0
Triceps	1	0
Heart + tongue	1	0
Liver +tongue	1	0
Lung +tongue	1	1(4.76)
Liver+heart +tongue	1	0
Masseter +tongue	3	2(9.52)
Tongue+massater+heart+liver	1	1(4.76)
Triceps +tongue	1	1(4.76)
Total	21	6(28.56)

IV. Discussion

In the present study, the overall prevalence of bovine cysticercosis among examined cattle in Mekelle Municipal abattoir was 6.7% which is comparable to the previous finding of (Getachew and Ashwani, 2008) in Mekelle (7.23%), (Nigatu, 2004) in Addis Ababa (7.5%), (Jemal and Haileluel 2011) in Kombolcha (6.66%),

(Dawit, 2004) in Gondar (4.9%) and (Haylegebriel and Alembrhan, 2012) in Eastern Tigray (5.73%).

However, report from this study was lower than the findings of Abuna*et al. (*2008) in Hawassa abattoir (26.25%); in North West Ethiopia (18.49%) by Kebede (2008); in east Shoa (17.5%) by Hailu (2005). It was also higher than the result of Tembo (2001) in central Ethiopia (3.2%) and Tekka (1997), in which the prevalence was 2.2%, (Tolosa*et al.* 2009) and(Gomol*et al,.* 2011) in Jimma municipal abattoir with prevalence of 2.93 % and 3.6%, respectively.

The above differences in prevalence of bovine cysticercosis might be due to personal and environmental hygiene variation in method and quality of meat inspection. Another possible reason for variation in prevalence may be due to difference in sample size, status of the people in the environment, the practical limitation to the number of incisions allowed in skeletal muscles, limit to the number and intensity of the incisions made during meat inspection (as this will reduce market price of the carcass) and the knowledge and ability of researchers as in agreement with (Jemal and Haleleul, 2011). Similarly management of animals, experience and diligence of inspector and inappropriate use of toilet in the area and dose and viability of eggs consumed by animals are some reasons for variation as suggested by Taresa *et al.* (2011). Time of occurrence could also contribute for the variation of prevalence in different studies (higher in dry season than rainy season) (Jemal and Haleleul, 2011).

In current study there was no statistically significant difference (P>005) in the prevalence of *C. bovis* infection between age and breed. It was concurred with earlier observation of (Hailu, 2005), Dawit (2004) and (Tembo, 2001) and was not in agreement with report of (Gomol*et al.,* 2011) and (Jemal and Haileleul, 2011). The possible explanation for this might be that any breeds of animal and age group have close susceptibility to Taeniasaginata. Additionaly, animals brought to the abattoir are in the same age group that means nearly adult and also the sample size is a factor for its insignificancy.

Neither sex nor, origin had statistical significance difference (p>0.05) with of animals associated with C. bovis infection. This was in line with the report of Gomol *et al.* (2011), Jemal and Haileluel (2011), (Dawit *et al*., 2012), Mesfin and Nuradddis (2012) and Haylegebriel and (Alembrhan, 2012). Nevertheless; this finding was in contrary to that of (Nuraddis and Frew 2012), who reported that statistically significant difference was observed between sexes of slaughtered animals. The likely reason for the non-significant difference between male and female slaughtered animals might be due to the fact that most of the animals brought to the abattoir had similar husbandry systems (the same type of livestock management) and both sexes were equally exposed to the disease in all districts, which leads to equal exposure of animals to *T. saginata* eggs.

Regarding the predilection sites of the cyst, the current finding revealed that the highest numbers of cysts were found on tongue followed by liver, masseter muscle and heart, triceps muscle and lastly lung in decreasing order. This finding was in agreement with the report of (Hailu 2005), (Solomon 2012), (Abunna*et al*., 2007) and Mesfin and Nuradddis (2012), who reported that tongue was the most frequently affected organ; all the parts of carcasses were equally important as predilection sites for cysticerci and could be equally used during routine meat inspection at slaughterhouses except for rumen, fat layers, spleen and skin. (Getachew and Ashwani, 2008).From several reports, variation and deviation in localization of cysts are quite possible. For example, (Dawit *et al*., 2012) and (Haylegebriel and Alembrhan, 2012) who reported that heart as being frequently affected by the cyst.

V. Conclusion and Recommendations

From the present study, it can be concluded that bovine cysticercosis was relatively higher prevalence in Mekelle and its adjoining areas and its prevalence was comparable to other parts of Ethiopia.Hence, bovine cysticercosisis is remaining as one of the major zoonotic diseases that can cause seriouspublic and socio-economic impact. Therefore; special attention should be given so as to prevent and control the spread of this disease in animals and in humans. The findings of the present study indicate that the health consequences of these zoonotic diseases and its economic impacts deserve serious attention by the various stakeholders, proper meat inspection and disposal of condemned organs in order to safeguard the well-being of the public. Moreover, establishment of policy on dog keeping and handling including registration, and treatment and elimination of stray dogs are essential.

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On Station Evaluation of Thermo-Stable Newcastle Disease Vaccine

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Keywords: *I2, ND, vaccination, and chicken.*

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I. Introduction

The fact that almost all of the poultry in Ethiopia comprises indigenous (i.e., local) birds reveals that the poultry subsector is strongly dominated by small-scale, household-level chicken production. According to Tadelle *et al*. (2003) and un-published document from National poultry research program, small-scale, village poultry production in Ethiopia contributes more than 98% of the national egg and poultry meat consumption, although this figure recently might have been changed slightly due to the emerging commercialization in peri-urban agriculture.

The indigenous flocks are said to be disease resistant and adapted to their environment. However, the survival rates of the Ethiopian indigenous chicks kept under natural brooding conditions considered low. Disease and predators are known to be the major causes of mortality in the country (Holye, 1992; Negussie, 1999). According to Negussie (1999), Newcastle disease accounted for the largest proportion of overall flock mortality to be 57.3% followed by fowl pox 31.6%, coccidiosis 9.4% and predator loss 1.7%.

In Ethiopia Newcastle disease (ND) appear to be the most challenging avian disease. The disease is capable of causing 90-100% mortality in unprotected flocks (Serkalem *et. al.,* 2005), (OIE, 2013). How the virus was introduced into the country is still unknown. The disease is transmitted from bird to bird and from farm to farm mainly via aerosol but contaminated feed and water, feces from sick bird and egg and carcass from infected birds are also means of transmission (OIE, 2012).

The common control strategies of the ND around the globe are vaccination, strict quarantine, slaughter and disposal of all infected and exposed birds and disinfection of the premises. Vaccination is generally very cost effective intervention and given a high priority by farmers in most developed nations where infrastructure and veterinary service are well known/available (Alexander *et al*., 2004).

Vaccination has a cost include price of the vaccine, time spent designing the vaccination schedule and paying for the crew that administers the vaccines. Another major cost for vaccination, which is rarely considered, is due to the losses from vaccine reactions from the live type vaccines and local tissue reactions associated with the inactivated vaccine injections (Dias *et al*., 2001). Many trials have been conducted to develop village vaccination program and reduce cost of Newcastle disease vaccination for scavenging poultry production system (Nasser *et.al.* 2010).

Having thermostable vaccine is enabling farmers to worry less on the logistics related with the cold chain. The immunogenicity of the thermostable I2 vaccine was mentioned by works of Nasser *et.al.* (2010) in ethiopia and Tu *et.al.* (1998) in Vietnam. Both report that chickens vaccinated with I2 vaccine with different route of vaccination and feed grains as a channel has a good protection effect. Many routes of vaccination were tested and their effectiveness is evaluated but the practicality of those vaccines in the country is not well disseminated in the farming community. This might be related with poor veterinary service and less practicality of the vaccination routes by rural community of the country. Therefore, the following trial on routes of vaccination was carried out in two breeds of chickens with the following objectives

a) Objective

- Determine the protection level of the litter based I2 thermo-stable Newcastle disease vaccine as compared to I2 in parboiled barley and water

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- Compare between the litter based I2 thermo-stable Newcastle disease vaccine systems with intra ocular.

II. Materials and Methods

a) Study Area

This experiment was conducted at Debre Ziet Agricultural Research Center (DZARC), Ethiopia.

b) Management of Experimental House and chickens

The experimental house and pens were thoroughly washed with water and sprayed with 3% of formalin. Separate pens were used for all treatment groups and control. After drying, clean new litter was spread over the floor. Equipments including waterier, feeders was cleaned, disinfected and introduced to the house. During brooding the room and brooder temperature was maintained with 250W infrared bulb per treatment group. Clean water and formulated feed was provided according to their requirement.

c) Experimental Design

The experiment used 190 local (Horo ecotype) and 295 KoeKoeK (South African breed) one day old chicks. The chicks were hatched at the Research center and proper hatching procedure was used. Ten sampled chicks were sacrificed and serum sample was collected at day one from both breeds. Blood sample was also collected again from 10 chicks at day 14 and 20 from both breed in order to get information on the level of maternal antibody. The remaining 180 chicks from local (indigenous) chicken and 285 chicks from koekoek breed were divided in to 15 equal groups. A single treatment has three replications. In this setup 4 treatments and 1 control were used. The sample size per treatment was calculated based on RCT sample size calculation (Chan 2003)

Table 1 : Experimental setup for I2 ND vaccination trial and viral challenge

RX	Breed	No. chicken	vaccinal viral dose	Days of vaccination*	Challenged chickens	challenge viral dose, (IM)
Eye-drop	Horro	36	10^6	21 & 36	10	10^9HA unit
	Koekeok	57	10^6	21 & 36	10	10^9HA unit
Water	Horro	36	10^6	21 & 36	10	10^9HA unit
	Koekeok	57	10^6	21 & 36	10	10^9HA unit
Feed	Horro	36	10^6	21 & 36	10	10^9HA unit
	Koekeok	57	10^6	21 & 36	10	10^9HA unit
Spray	Horro	36	10^6	21 & 36	10	10^9HA unit
	Koekeok	57	10^6	21 & 36	10	10^9HA unit
Naive	Horro	36	10^6		10	10^9HA unit
	Koekeok	57	10^6		10	10^9HA unit

d) Vaccination

The experiment uses different route of vaccination on the four treatment groups. The treatments mentioned below were given twice in 15 days interval at day 21 and 36. The vaccine was purchased from National Veterinary Institute. The vaccination was carried out after 3 weeks of age to override effect of maternal immunity (Nasser. *et al.,* 2010).

Eye vaccination: individual chicken base one eye one drop method was used to vaccinate though eye drop. A sterile standard pastor pipette was used.

Water vaccination: A water vaccination is given to chicken using distilled water. The chicks were kept without water for 2.5 hours prior to vaccine administration. It uses 10 ml per bird in the first vaccination and 20 ml per bird in the second vaccination (NVI manual).

Feed vaccination: Parboiled barley preparation was adopted from Nasser *et al.* (2010). One kg of grain is added to 1.75 litres of boiling water and left for 5 minutes. It was cooled using water. The grain was then sun dried and cracked manually. Then 1 kg with 4 liters of water twice in a day and leave it soaked overnight.

Then dried it using sunlight and use it for the treatment. The prepared barley was then sprayed using a fine sprayer in the ratio of 1 ml per 10 gram of grain. The feed was given to the birds by calculating 10 gram of feed per bird. This shows that the dose of the virus required for a single bird was calculated per 1 ml of the reconstituted vaccine.

Litter spray vaccination: I2 vaccine was used to spray the litter where experimental chicken were kept. A 1 ml per bird ratio was used in each breed.

e) *Serum Collection and Haem-agglutination Inhibition Test*

Blood sample was collected at day 1, 14, 20, 36, 44, 51, 58, 65 and 82 before and after vaccination. In average 1-2 ml of blood was collected in each bleeding days. Scarification was used in DOC but jugular vein and brachial vein were used in other age groups to take blood. The collected whole blood was labeled and allowed to clot under normal atmospheric condition. Then, the clear serum was harvested into labeled cryovials and stored at -20°C until HI test was carried out. The challenge virus was administered at day 65. Post challenge bleeding was done on survivors of the deadly velogenic viral challenge. The collected sera at pre and post vaccination and post challenge were tested using heamagglutination inhibition test. The test was performed following the method described in OIE (2009) manual for hemaglutination and inhibition test and the protocol of national veterinary institute (NVI). The antibody level for each serum sample was recorded using well designed recording sheet.

f) *Challenge with Virulent Field Virus*

Wild virus was collected from chicken embryo at NVI vaccine quality laboratory. The wild virus which collected from Haromaya by NVI was tested for haem-agglutination before administration to the birds in order to check its potency. Ten chickens from each treatment was isolated and challenged at day 65 with wild strain of ND virus. The challenge viral dose was in accordance with the work of Darminto and Daniels (1992) and Khalafall *et. al.,* (2004). The virus was given via Intra Muscular route in the breast muscle (Khalafall *et. al.,* 2004) and (Nasser *et al.*, 2010). The birds were kept under close observation for 15 days. Numbers of dead and live birds was recorded. Standard bio-security measures like restriction of movement, proper disinfection and disposal of dead chicken were implemented in-order to prevent the spread of disease.

g) *Pathogenicity Index Measurement*

The pathogenicity index for the challenge virus was measured using tools adopted from Tizard, 2004. The pathogenic index was set based on the time taken until an event is occurred in individual animal. To follow individual chicken each chicken was wing tagged. Then the chickens were followed for 15 days and occurrence of an event is recorded. For pathogenic index measurement four (4) categories were used according to Tizard (2004) and Mishra *et al.*, (2001). Category 0 (Zero) was given to the chicken when there was no any clinical signs; 1 (one) for in appetence and depression, 2 (two) for discharges and nervous signs, and 3 (three) for death.

III. Results

a) *Hi titter in experimental animals*

The result shows that there was no a significant difference in the antibody response between breeds (Table 2). But there was a significant difference between treatments (table 3).

Table 2 : Protective HI titter between two breeds

	Sum of Squares	Df	Mean Square	F	P
Between Groups	7.202	1	7.20	2.25	.134
Within Groups	1333.795	418	3.19		
Total	1340.998	419			

Table 3 : HI titters between treatments after vaccination

Treatment (1)	Treatment (2)	Mean Difference (1-2)	Std. Error	Sig.
Ocular	Water	18.889*	5.157	.003
	Feed	18.444*	5.157	.004
Water	Feed	-.444	5.157	1.000
Spray	Ocular	-3.056	5.157	1.000
	Water	15.389*	5.157	.030
	Feed	15.833*	5.157	.023
	Naïve	43.341*	4.969	.000
Naïve	Ocular	-46.397*	4.969	.000
	Water	-27.508*	4.969	.000
	Feed	-27.952*	4.969	.000

b) *Pathogenic index*

There is no significant difference (P= 0.82) in the pathogenic index between breeds but there is significant difference between treatments. The result shows control groups were the first in exhibiting the disease outcome in shorter time than the other four treatments.

Table 4 : Mean Pathogenic Index of chickens under each treatment

Treatment	Mean	N	Std. Deviation
Ocular	0.18	20	.554
Water	0.74	20	1.122
Feed	1.35	20	1.105
Spray	0.42	20	.860
Naïve	2.43	20	.233
Total	1.02	100	1.159

Chickens in all treatments have significantly lower pathogenic index than that of chicken in control group. The pathogenic index is not significantly different between birds' vaccinated using spray, water and ocular route of vaccination but it is significantly lower in chicken vaccinated with barley (Table. 18)

The pathogenic index of the challenge virus in the four treatment groups is not significantly different in the two breeds of chicken. This shows that the breed effect on the pathogencity of the disease is not significant.

c) Survival rate of chicken after challenge

The result from this study revealed that the survival rate of the chicken after wild ND viral challenge was higher in all treatments than the control group, but between breed difference in the survival of the field challenge is not significantly different (P=0.6).

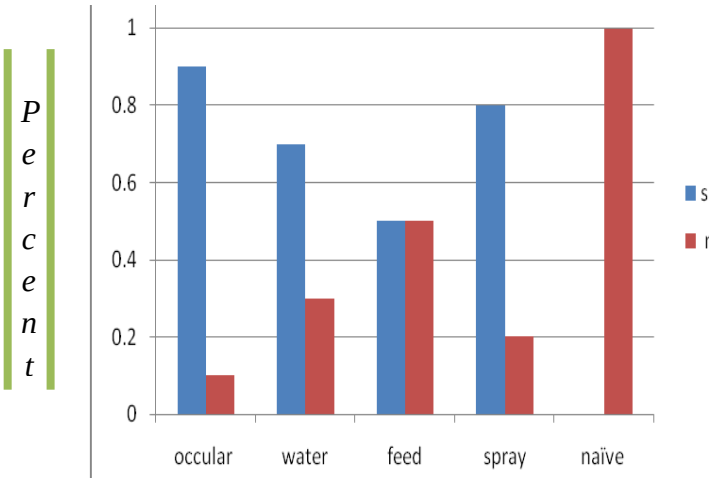

Fig. 1 : Post challenge percent survival of chicken in the five treatment groups (blue= survive & Red= dead)

Survival probability of chicken in different treatments groups is measured using mortality probability of chicken with relation to their HI titter.

d) Relationship between mean HI titer and mortality of experimental chickens

The picture below shows the mean titter of HI for the four treatments with relation to the control group is high. The result shows that the loss related with Newcastle disease in unprotected flock was up to 100%.

Table 5 : Pathogenic index difference of different treatments

Rx	Rx	M D	S. E	Sig.	95% C I	
					LB	UB
water	Eye	.559	.268	.234	-.19	1.30
Feed	Eye	1.167*	.268	.000	.42	1.91
	Water	.609	.268	.163	-.14	1.35
Spray	Eye	.236	.268	.903	-.51	.98
	Water	-.322	.268	.748	-1.07	.42
	Feed	-.931*	.268	.007	-1.68	-.19
Control	Eye	2.250*	.268	.000	1.51	2.99

Water	1.691*	.268	.000	.95	2.44
Feed	1.083*	.268	.001	.34	1.83
Spray	2.014*	.268	.000	1.27	2.76

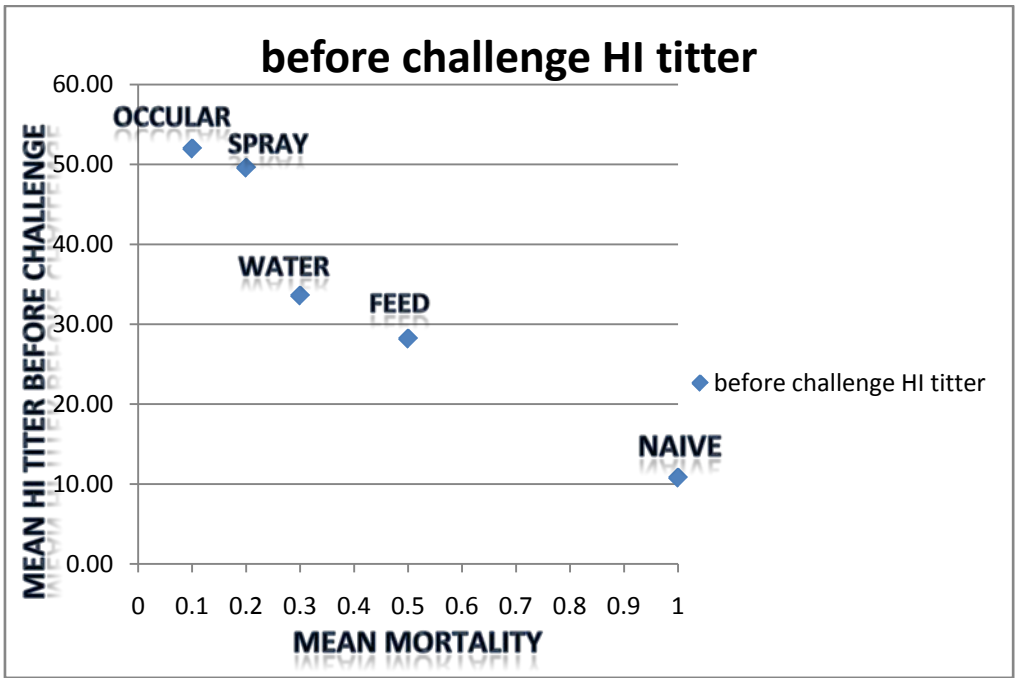

Fig. 2 : Mean HI titter before the challenge

The survival time of chicken after the challenge is lower in control group than any other treatments. The survival time significantly higher in chicken in chickens under ocular and spray route of vaccination. Survival rate of chickens which takes vaccine through vaccine treated feed is relatively lower than other treatment groups.

The survival curve showed that more than 75% of the chicken in the ocular, spray and water treatment groups survived the mortality. The survival rate of chicken in the control treatment was zero percent after 8 days post challenge (Figure 3).

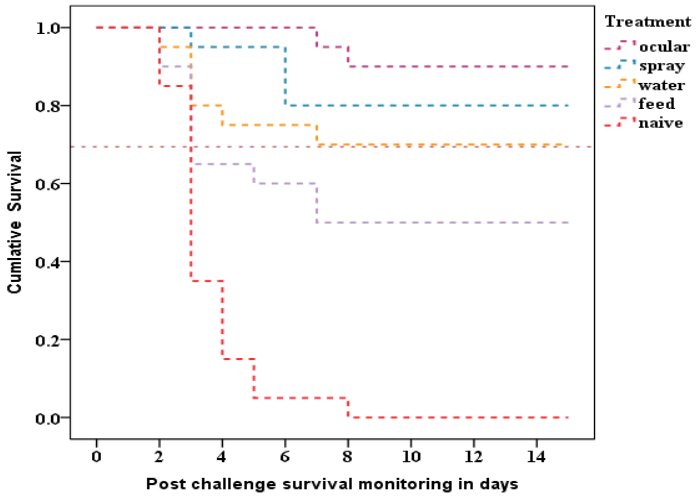

Fig. 3 : Post challenge survivality of chicken

IV. Discussion

The protection level of the vaccine in the naive treatment groups in this study was in agreement with Musa *et al.* (2010) as the HI titter of chickens that were not taking no vaccination have unprotected antibody titter. The high mortality of chicken in naive group had similarity trend with control groups (naïves) of other

works and literatures elsewhere (OIE, 2013), (Hussain *et al.,* 1988) and (Nasser *et al.,* 2010). According to the FAO, 2005 and manual by OIE, 2013; NDV can cause 100% mortality in unprotected flock devastating outbreak condition.

In study which performed using bran, ground grain and water as a vehicle by (Abdu *et.al,* 2012); water vaccination was more protective than vaccination using feed as a channel. The difference in the immune response of chicken on vaccinated with water and feed is the time taken to take the formulated vaccine. It is taking longer in feed channel than that of water. This is mainly related with inadaptability of the chicken for the feed that the vaccine is constituted. It is believed that prior adaptation for the grain in which that vaccine was given may be increase the efficiency of the vaccine. Study by Musa *et al,* 2010; the mortality of chicken that were vaccinated with vaccine treated sorghum is devastating (up to 100% mortality), this is different from the result of the current study. The finding of this study on treated barley is different from the findings of Nasser *et al.,* (2010) which report more than 90% protection. This might be due to the number of animal under the challenge and the difference in the type of chicken used in the treatment. Broiler chicken was used by Nasser *et al.,* (2010) and according to Mozaffor *et al.,* (2010) broiler chicken have higher sero conversion for Newcastle disease than that of layer chickens.

In this investigation, better results were obtained when chicks were vaccinated via eye drop and litter spray route. This agreed with the findings of vaccination trials conducted in other African countries, using the same or other thermostable vaccines of ND (Musa *et al,* 2010; Hussain *et al.,* 1988, Foster *et al,* 1997, Khalafall *et al.,* 2004). On the other hand, chicks vaccinated by water showed remarkably lower immune responses and protection rates as compared to ocular and spray vaccination, but higher than that vaccinated with feed as a channel. The reduced response of the birds to vaccines that are given by oral routes is mainly due to virus viability be lost at the gastrointestinal tract (GIT), unless high amount of NDV is contained in the vaccine (Shuaib *et al.,* 1985). It is also reported on Spradbrow (1992) that the viral load excreted from orally vaccinated chicken was little or zero after the second vaccination when faecal extracts possessed neutralizing activity, probably associated with IgA antibody.

Based on these findings, the intra ocular rote administration of I2 vaccine is recommended for the vaccine application especially for village chickens where number of chickens in a flock is small. However, to implement conventional vaccination methods chickens are difficult to catch which is also reported by Latif *et.al.,* (1992). But spray vaccination which can be performed by middle level professional easily is a simple means of vaccinating chicken. Following the virus administered by spray it follows the natural route of infection, it reaches the upper respiratory tract through the naso-lacrymal duct where it multiplies to induce the required immune responses. This technique can also be practical on commercial production system that has large numbers of chickens to be immunized all at once.

V. Conclusions and Recommendation

ND is responsible for massive rural chicken loss that makes farmers to loss their trust in poultry production as a means to alleviate poverty and improve family nutrition. The current experimental ND vaccination trial of this study provides an alternative vaccine administration routes. This will contribute in researches and targets towards prevention and control of ND disease. This is believed to have a potential for significant improvement in the livelihood of poor people. Accordingly, protection level of intraocular and spray vaccination is better than that of water and feed vaccination. However, the litter spray route is the easiest, affordable and highly effective means of vaccinating village chickens.

Based on the above conclusion the following recommendations were forwarded: Newcastle disease prevention and control with routine vaccination program should be of the first priority in village production system. Among the vaccination routes tested in this experiment, litter spray vaccination of thermo-stable vaccine is the preferable one for scavenging small scale production system where ocular vaccination application is very difficult. In addition for proper vaccination of chickens, training on management practices; village bio-security and nutrition must be implemented with. To complete the output of this result, on farm evaluation of ND vaccination and training of farmers in the study area should be implemented and the effect of the intervention should be quantified.

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TECHNIQUES FOR WRITING A GOOD QUALITY RESEARCH PAPER:

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2. Evaluators are human: First thing to remember that evaluators are also human being. They are not only meant for rejecting a paper. They are here to evaluate your paper. So, present your Best.

3. Think Like Evaluators: If you are in a confusion or getting demotivated that your paper will be accepted by evaluators or not, then think and try to evaluate your paper like an Evaluator. Try to understand that what an evaluator wants in your research paper and automatically you will have your answer.

4. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

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6. Use of computer is recommended: As you are doing research in the field of Computer Science, then this point is quite obvious.

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9. Use and get big pictures: Always use encyclopedias, Wikipedia to get pictures so that you can go into the depth.

10. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right! It is a good habit, which helps to not to lose your continuity. You should always use bookmarks while searching on Internet also, which will make your search easier.

11. Revise what you wrote: When you write anything, always read it, summarize it and then finalize it.

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21. Arrangement of information: Each section of the main body should start with an opening sentence and there should be a changeover at the end of the section. Give only valid and powerful arguments to your topic. You may also maintain your arguments with records.

22. Never start in last minute: Always start at right time and give enough time to research work. Leaving everything to the last minute will degrade your paper and spoil your work.

23. Multitasking in research is not good: Doing several things at the same time proves bad habit in case of research activity. Research is an area, where everything has a particular time slot. Divide your research work in parts and do particular part in particular time slot.

24. Never copy others' work: Never copy others' work and give it your name because if evaluator has seen it anywhere you will be in trouble.

25. Take proper rest and food: No matter how many hours you spend for your research activity, if you are not taking care of your health then all your efforts will be in vain. For a quality research, study is must, and this can be done by taking proper rest and food.

26. Go for seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

27. Refresh your mind after intervals: Try to give rest to your mind by listening to soft music or by sleeping in intervals. This will also improve your memory.

28. Make colleagues: Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

29. Think technically: Always think technically. If anything happens, then search its reasons, its benefits, and demerits.

30. Think and then print: When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

31. Adding unnecessary information: Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

32. Never oversimplify everything: To add material in your research paper, never go for oversimplification. This will definitely irritate the evaluator. Be more or less specific. Also too, by no means, ever use rhythmic redundancies. Contractions aren't essential and shouldn't be there used. Comparisons are as terrible as clichés. Give up ampersands and abbreviations, and so on. Remove commas, that are, not necessary. Parenthetical words however should be together with this in commas. Understatement is all the time the complete best way to put onward earth-shaking thoughts. Give a detailed literary review.

33. Report concluded results: Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

34. After conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print to the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects in your research.

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Key points to remember:

- Submit all work in its final form.
- Write your paper in the form, which is presented in the guidelines using the template.
- Please note the criterion for grading the final paper by peer-reviewers.

Final Points:

A purpose of organizing a research paper is to let people to interpret your effort selectively. The journal requires the following sections, submitted in the order listed, each section to start on a new page.

The introduction will be compiled from reference matter and will reflect the design processes or outline of basis that direct you to make study. As you will carry out the process of study, the method and process section will be constructed as like that. The result segment will show related statistics in nearly sequential order and will direct the reviewers next to the similar intellectual paths throughout the data that you took to carry out your study. The discussion section will provide understanding of the data and projections as to the implication of the results. The use of good quality references all through the paper will give the effort trustworthiness by representing an alertness of prior workings.

Writing a research paper is not an easy job no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record keeping are the only means to make straightforward the progression.

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To make a paper clear

· Adhere to recommended page limits

Mistakes to evade

- Insertion a title at the foot of a page with the subsequent text on the next page
- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

· Use standard writing style including articles ("a", "the," etc.)

· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

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The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing references at this point.

An abstract is a brief distinct paragraph summary of finished work or work in development. In a minute or less a reviewer can be taught the foundation behind the study, common approach to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

- Reason of the study - theory, overall issue, purpose
- Fundamental goal
- To the point depiction of the research
- Consequences, including definite statistics - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

Introduction:

The **Introduction** should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

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- Sort out your thoughts; manufacture one key point with every section. If you make the four points listed above, you will need a least of four paragraphs.

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This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not particulars of each process that engaged the same methodology)
- Describe the method entirely
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

What to keep away from

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings - save it for the argument.
- Leave out information that is immaterial to a third party.

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The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.
- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
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- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report
- If you desire, you may place your figures and tables properly within the text of your results part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
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- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

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The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
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- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
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- Submit to generally acknowledged facts and main beliefs in present tense.

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Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

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