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Evaluation of Sensory Silage Quality, Chemical Composition and in vitro Digestibility of Tef (*Eragrostis tef*) Straw Inoculated with Effective Microorganisms (EM) at different Application Rates and Ensiled for different Durations

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ABSTRACT

Background: Ethiopian livestock production is suffering from feed scarcity and low quality. Enhancing the nutritive value of the available poor quality feed resources like tef straw (TS) is believed to improve feed quality and supply. However, there were no studies done on improvement of TS with the use of effective microorganisms (EM). Hence, the study was conducted with the objective of assessing the effect of application rate (AR) and duration of ensiling period (EP) of fermentation of tef straw (TS) with EM on silage sensory quality, dry matter (DM) and nutrient composition and in-vitro digestibility.
Methods: The experimental design was completely randomized design with three replications and two 3 x 3 factor (three (EM) application rates and three ensiling periods). The EM stock solution was used for one month after activation and dilution. The silage was made from TS soaked in water overnight, inoculated with EM and packed in plastic bags. At the end of ensiling period the bags were withdrawn and the silages were subjected to physical appraisals, pH measurement, chemical and in-vitro digestibility analysis.
Results: The physical appraisal of all the silages were nearly similar and of good quality types. However, the pH was statistically not affected by ensiling period and not sufficiently low. But, this was not reflected in the nutrient composition of the silage as the fermented TS ensiled for 21 days contained higher crude protein and organic matter. The same was true for in-vitro organic matter digestibility. EM application rate did not affect digestibility and nutrient composition except the ash and organic matter content.
Conclusion: cold water socked TS ensiled for 21 days inoculated with EM at a rate of 500mL/kg was found to have better nutritional quality than those ensiled for 14 and 28 days at rates of 250 and 750mL/kg application rates.

1. INTRODUCTION

Animal production in Ethiopia has been on a very low profile due to chronic shortage of feed. Scarcities of feeds and under nutrition continue to cause low performance of animals. The major feed resources are crop residues (including *tef* straw) in mixed farming areas and degraded range grazing and standing hay in pastoralist areas (Alemayehu et al 2017; Mekuanint and Girma, 2017) which can't fulfill even the maintenance requirement of the animals (Adugna et al 2012;Malede and Takele, 2014).

The ever-increasing human population has also caused conversion of range/pasture lands into croplands leading to diminishing land available for grazing and fodder production. On the other hand, livestock, especially cattle population has been increasing to meet the demand for power to the increased crop production activity, while the quality and quantity of forage available from natural pasture is declining due to excessive grazing pressure (Adugna et al 2012; Malede and Takele, 2014). Hence, focusing on options other than increasing the size of grazing land, to address the feed availability and quality challenges is of great necessity.

Improving the nutritive value of the poor quality feed resources is one option of improving feed quality and supply. Biological treatment of low quality roughage is one of the effective feed treatments technologies (Abdel-Aziz et al, 2015; Mahesh and Mohini, 2013). In this regard, the use of Effective Microorganisms (EM) is widely advocated for improving nutritional qualities (Wondmeneh et al 2011; Samsudin et al 2013; Yonatan et al 2013). EM is a product characterized by a mix of aerobic and anaerobic microorganisms in particular, lactic acid bacteria, yeast, and photosynthetic bacteria, fermenting fungi and actinomycetes (Higa and Wididana, 1991, Talaat et al 2015). These microorganisms assist one another for survival in a food chain and thereby form a synergy that fights off pathogens and rotting microorganisms. The bacteria producing lactic acid inhibit the growth of pathogenic microorganisms by reducing the pH. Yeast produces many substances like amino acids and polysaccharides which are food for other microbe while the phototrophic bacteria are involved in various metabolic systems playing important role in nitrogen and carbon cycles (Higa and Wididana, 1991, Talaat et al 2015).

Lemma and Endalew (2017) found that *Fogera* cows fed EM treated rice straw showing higher dry matter intake, higher weight gain and higher milk yield. Getu et al (2013) also noted that cows receiving EM in the form of Bokashi displayed unusually superior daily growth over those cows that have been dosed with EM through the sprayed natural pasture hay. However, there are no studies done to evaluate the effect of application rate of EM and ensiling period on *tef* straw (TS).

Hence, the present study was conducted with the objective of assessing the effect of application rate (AR) and ensiling period (EP) of TS with EM on silage **sensor** quality, dry matter (DM) and nutrient composition and *in-vitro* digestibility.

2. MATERIALS AND METHODS

2.1 Activation and extension of effective microorganism solution

The EM stock solution was purchased from Woljjejji private limited company and used after activation and subsequent extension following the user manual of the company. Accordingly, activated EM solution was prepared by mixing (thoroughly stirred until uniform solution is obtained) EM stock solution, molasses, mango juice and chlorine free water in the ratio of 1:1:1:18 and then storing in an airtight container for 21 days. The resulting solution is now activated EM solution which can be used for one month. The activated EM solution was used after it was diluted by mixing (thoroughly stirring until uniform solution is obtained) activated EM solution, molasses and chlorine free water in the ratio of 1:1:18 and then storing it in an airtight container overnight. Molasses was added into the EM solution in order to initiate the microbial metabolism and proliferation. Cane molasses has brix (degree brix), dry suspended particles (>100µ), total sugars as invert, calcium as Calcium oxide, reducing sugars, nitrogen content, sulphated ash and unfermentable sugars % w/w 86, 13.27, 41.6, 1832.64, 12.5, 0.90, 17.12 and 5.55, respectively (Fekadu, 2007).

2.2 Ensiling procedure and experimental treatments

The TS was purchased from localities around Bishoftu town. FTS was prepared by uniform spraying of water over TS layer by layer at a rate of 1:2 (volumes by mass) in an earth silo covered with thick plastic sheath. Then the wetted TS was fully covered with the plastic sheath and left overnight being pressed by heavy weight distributed uniformly over its top so that the water is fully and uniformly absorbed. The next morning, the wetted TS was thoroughly mixed and uniformly sprinkled over by the extended activated EM solution and packed tightly in an air tight plastic bag and stored under shade.

The experimental design used was completely randomized design with three replications per treatment and two 3 x 3 factors (Table 1), namely three EM application rates (250, 500 and 750 mL EM/1 kg dry TS (AR_{250}, AR_{500} and AR_{750} respectively)) and three durations of ensiling period (14, 21 and 28 days (EP_{14}, EP_{21} and EP_{28}, respectively)).

Table 1: Experimental treatments of fermenting *tef* straw

Pre-treatment action[a]	EM[b] application rate (AR, mL EM/kg dry TS)	Ensiling period (EP, days)		
		EP_{14}	EP_{21}	EP_{28}
Overnight water socked	250	14	21	28
Overnight water socked	500	14	21	28
Overnight water socked	750	14	21	28

[a]teff straw was socked with cold water with the same rate of water : dry(as is) tef straw of 1:2 (Litter by kg)
[b]EM=effective micro-organisms

At the end of an ensiling period, the bags meant for the period were withdrawn and samples of the FTS were taken from each bag and stored frozen (-18^{o}C) until prepared for chemical analysis while the sensory analysis and pH measurement were performed just during withdrawal of the bags.

2.3 pH measurement and sensory evaluation of samples of fermented *tef* straw

The pH and sensory feed quality evaluation was performed at the end of each experimental ensiling period. The pH measurement was done using portable digital pH meter (HI99163, HANAN instruments) while the sensory evaluation (color, odor, texture and fungal prevalence) was performed subjectively by a test panel of eight people which were given a sort of training for silage sensory appraisal.

A sample from each ensiling bag was collected by untying and making tiny opening through which the sample was taken from the top, middle and bottom using forceps. The pH of samples was measured according to Playne and McDonald (1996). Consequently, 20 g of samples were put in plastic containers having firm cap into which 100mL of distilled water was poured. Then, the container was firmly fitted with the cap and stored for 24hrs. Next day, each container was shacked thoroughly and extracts were filtered and collected in three equal plastic cups from which the pH was measured. The odor, color, texture and fungal prevalence of samples were assessed just after opening the silage bags.

During the sensory evaluation, the panelists (assessors) were offered color chart to which they compare the color of the samples and assign color name. The assessors also name the odor of the samples by relating it to the usual odor of alcohol, yogurt, fresh local cheese, hot bread, vinegar, burnt tobacco, fishy or deviating from these odors. For the prevalence of fungus they simply see for the presence of mold. The assessors take a look to the ensiling bags by walking around in random order with no opportunity to see each others' judgment. They were provided with record sheet for scoring their assessment and the highest frequently scored judgment of the assessors was taken as the value of the assessment for each sensory parameter.

2.4 Sample preparation for chemical analysis

To prepare the samples for analysis, they were thawed to room temperature overnight after which they were oven dried at 60^{o}C for 48 hours upon which partial dry matter was determined. The oven dried samples were then ground through 1 mm sieve and stored in airtight polyethylene containers until analyzed. Finally, the samples were subject to proximate (AOAC, 1990), detergent fibers (Van Soest et al 1991 and Van Soest and Robertson, 1985) and *In vitro* digestibility (Tilley and Terry, 1963) analysis.

2.5 Chemical analysis

Dry matter and nutrient composition except detergent fibers were determined in the nutrition laboratory of national veterinary institute. The *in vitro* digestibility was determined in *Holeta* agricultural research center while the detergent fibers were analyzed in Haramaya University animal nutrition laboratories. DM was determined by drying samples overnight at 105^{o}C in a forced draft oven. Ash was determined by combusting sample at 550^{o}C for 6 hours and organic matter (OM) was determined by difference as 100-ash%. CP was computed from the respective N contents as determined by the Kjeldahl method employing the equation CP = N x 6.25 (AOAC, 1990). Neutral detergent fiber (NDF) was analyzed using the procedures of Van Soest et al (1991) whereas acid detergent fiber (ADF) and acid detergent lignin (ADL) were determined according to Van Soest and Robertson (1985). Ether extract was determined by extracting the ether soluble components through continuous evaporation and condensation. The extracted material was then dried and weighed. Metabolizable energy (ME) was estimated according to McDonald et al (2010) from the *in-vitro* digestible organic matter in DM (IVDOMD, g kg^{-1} DM) which is the product of the OM content (g kg^{-1} DM) and IVOMD coefficient of the feed as: [ME (MJ kg^{-1} DM) = 0.016 IVDOMD].

2.6 Statistical Analysis

Data on nutrient composition, in-vitro digestibility and pH of fermented tef straw were analyzed using JMP™, The Statistical Discovery Software™ Version 5 and mean

differences were tested using LSMean Tukey HSD mean separation tool (SAS, 2002) and considered significant at $p<0.05$.

The model used was $\mathbf{Y_{ijk}= \mu + AR_i + EP_j + (AREP)_{ij} + e_{ij}}$; Where: $\mathbf{Y_{ij}}$ = Response variable; $\boldsymbol{\mu}$ = mean of the population; $\mathbf{AR_i}$ = the $\mathbf{i}^{th}$ EM application rate effect; $\mathbf{EP_j}$ = $\mathbf{j}^{th}$ ensiling period effect; $\mathbf{(AREP)_{ij}}$ = the effect of interaction between $\mathbf{i}^{th}$ EM application rate and $\mathbf{j}^{th}$ ensiling period; $\mathbf{e_{ij}}$ = random error

3 RESULTS

3.1 Sensory evaluation of samples of fermented *tef* straw

The sensory appraisal of fermented *tef* straw treated with EM at different application rate and ensiled for varying duration is presented in Table 2. All the FTS silage did not show any sign of mold development.

The color of the silage ranges from banana (light yellow) of TS ensiled for 14 day with 250 mL EM application to mustard (dark yellow) of TS ensiled for 28 day with 500 and 750 mL EM application rate. This confirmed that the color of the silage tended to change from light to dark with increasing rate of EM application and ensiling duration.

It was also observed that all the silages tended to get darker upon exposure to air with no change in odor. There was no foul smell felt, but pleasant acid smell varying from very weak yogurt smell of TS ensiled for 14 day with 250 mL/kg EM application rate to weak cheese for TS ensiled for 28 day with 500 and 750 mL/kg EM application rate.

The silage of TS ensiled for 21 days at the EM application rate of 500 mL/kg situated in between the two extremes for both color and odor as it has shown darker yellow (tuscan sun) color and good yogurt odor.

Table 2: Sensory appraisal of fermented *tef* straw treated with EM at three application rates and three ensiled durations

Ensiling period (EP)	EP_{14}			EP_{21}			EP_{28}		
EM application rate (AR)	AR_{250}	AR_{500}	AR_{750}	AR_{250}	AR_{500}	AR_{750}	AR_{250}	AR_{500}	AR_{750}
Color	banana	blond	tuscan sun	blond	tuscan sun	mustard	blond	tuscan sun	mustard
Odor	very weak yogurt	weak yogurt	weak yogurt	weak yogurt	good yogurt	sour yogurt	good yogurt	sour yogurt	weak cheese
texture	flexible & none sticky stems	flexible & none sticky stems	flexible & none sticky stems	flexible & none sticky stems	flexible & none sticky stems	flexible & none sticky stems	flexible & none sticky stems	flexible & none sticky stems	flexible & none sticky stems
mould	none	none	none	none	none	none	none	none	none

AR=application rate; AR_{250}=250mL EM: 1kg *tef* straw; AR_{500}=500mL EM:1kg *tef* straw; AR_{750}=750ml EM: 1kg *tef* straw; EM=Effective microorganism; EP=ensiling period; EP_{14}=14 days ensiling period; EP_{21}=21 days ensiling period; EP_{28}=28 days ensiling period.

The odor of the silage as the case of their color also showed clear gentle linear pattern of change from very weak yogurt to weak cheese with increasing rate of EM application and ensiling duration. The texture of all the FTS silage was found to have soft stems which were none slimy and separable upon chafing between fingers.

3.2 Nutrient composition, *in-vitro* digestibility and pH of fermented *tef* straw

The nutrient and estimated ME composition, *in- vitro* DM and OM digestibility and pH of FTS is summarized in **Table 3**. Duration of the ensiling period did not affect the pH, nutrients and ME composition, except IVOMD, and OM and CP contents.

The OM and CP content of FTS under EP_{21} were similar to that of under EP_{14} and higher ($p<0.05$) than that of under EP_{28} whereas the IVOMD of FTS under EP_{21} was higher ($p<0.05$) than that of under EP_{14} and comparable with that of under EP_{28}. Similarly, the effect of EM application rate was significant ($p<0.05$) only for pH value, ash content and OM content of FTS.

The ash content was higher ($p<0.05$) in the FTS under AR_{750} than FTS under the other two ARs, which were not statistically different from each other. The OM contents of the FTS under AR_{250} and AR_{500} were not different to each other but, higher ($p<0.05$) than that of under AR_{750}.

The pH of the FTS under AR_{750} was more acidic than under AR_{250} but that of under AR_{500} was not different from both under AR_{250} and AR_{750}. The effect of interaction between the duration of ensiling period and

EM application rate were not presented as they were not significant.

Table 3 **Dry matter (%), nutrient (g kg^{-1}DM), estimated ME(MJ kg^{-1} DM) composition, *In vitro* OM digestibility (%) and pH of treated *tef* straw in fermentation experiment**

Variable[e]	Ensiling period (EP)[f]			SEM[f]	Application rate (AR)[f]			SEM[f]	p-value		
	EP_{14}	EP_{21}	EP_{28}		AR_{250}	AR_{500}	AR_{750}		EP	AR	EP x AR
DM	33.97	32.95	34.39	1.49	36.16	33.10	32.04	1.49	0.78	0.16	0.68
Ash	78.95^{ab}	77.46^{b}	80.72^{a}	0.74	76.78^{y}	78.11^{y}	82.23^{x}	0.74	0.02	0.0002	0.14
OM	921.07^{ab}	922.54^{a}	919.27^{b}	0.75	923.22^{x}	921.90^{x}	917.78^{y}	0.75	0.02	0.0002	0.14
CP	54.65^{ab}	58.02^{a}	51.58^{b}	2.70	54.81	55.52	53.92	1.60	0.01	0.24	0.09
EE	11.41	10.13	9.45	0.69	10.59	10.32	10.09	0.69	0.15	0.88	0.47
NDF	281.93	269.49	276.63	12.83	296.56	268.29	263.19	12.83	0.79	0.17	0.78
ADF	150.22	135.03	154.77	6.53	154.89	143.25	141.87	6.53	0.11	0.32	0.83
ADL	56.79	46.61	64.88	5.75	56.65	60.25	51.38	5.75	0.11	0.56	0.07
IVDMD	43.29	41.86	41.21	0.80	42.82	42.48	41.07	0.80	0.20	0.29	0.64
IVOMD	37.43^{b}	40.02^{a}	38.28^{ab}	0.68	37.95	38.97	38.80	0.68	0.04	0.56	0.63
IVDOMD	344.78^{b}	369.13^{a}	351.82^{ab}	6.81	350.33	359.26	356.14	6.12	0.04	0.54	0.64
ME	5.52	5.90	5.63	0.10	5.60	5.75	5.70	0.10	0.22	0.41	0.65
pH	4.76	5.22	4.91	0.24	5.67^{x}	4.99^{xy}	4.24^{y}	0.24	0.41	0.002	0.08

[e]ADF=Acid detergent fiber; ADL=Acid detergent lignin; CP=Crude protein; DM=Dry matter; EE=Ether Extract; IVDMD= *in vitro* DM digestibility; IVOMD=*in vitro* OM Digestibility; IVDOMD (g kg^{-1}DM)=*in vitro* digestible OM in DM;OM=Organic matter; ME=Metabolizable energy; NDF=Neutral detergent fiber.

[f]AR_{250}=250mL EM:1kg *tef* straw; AR_{500}=500mL EM:1kg *tef* straw; AR_{750}=750ml EM: 1kg *tef* straw; EM=Effective microorganism; EP_{14}=14 days ensiling period; EP_{21}=21 days ensiling period; EP_{28}=28 days ensiling period; SEM=standard error of mean; Values in a row superscribed by different letters are significantly different, letters ^{a,b,c} standing for EP and ^{x,y,z} standing for AR.

4 DISCUSSION

4.1 Sensory evaluation of samples of fermented *tef* straw

There is shortage of literature on *tef* straw silage treated with EM or other microbial inoculums. But, from general straw silage characteristics it can be observed that, regardless of the EM application rate and the ensiling duration, the TS silage in the present study was of good quality category as it has maintained the color of the dry straw with minimum change (Kaiser and Piltz, 2003).This is confirmed by its good yogurt smell indicating that lactic acid was sufficiently produced by the fermentative action of lactic acid bacteria in the EM solution (Kim et al 2017;Higa and Wididana, 1991; Zhang et al, 2016). Furthermore, the absence of mould, ill color and bad smell might have been attributed to the dominance of the desirable over undesirable microorganisms (Kaiser and Piltz, 2003; Oladosu et al, 2016).

4.2 Nutrient composition, *in-vitro* digestibility and pH of fermented *tef* straw

Daniel et al (2016)obtained 4.17 pH in EM fermented chopped sorghum stover at a rate of 950 mL/kg DM and ensiled for 21 days which is relatively similar to the 4.24 pH value of the FTS ensiled for 28 days with EM application rate of 750 mL/kg in this study. The slight difference seen between the two studies could be attributed to the difference in the substrate (TS *vs* sorghum stover), the EM application rate and ensiling duration.

There is general agreement that a silage pH below 4.5 is an indicator for the occurrence of adequate anaerobic fermentation that increases hydrolysis of fiber fractions of the feed (FAO 1999). Accordingly, the results of the current study indicate that the TS ensiled with the EM application rate of 750 mL/kg could be categorized as good silage as itspH values was less than to4.5.

In support of the present study, Samsudin et al (2013) reported significant effect of EM inoculation of fungal-treated rice straw on CP and OM content. Conversely, they also found a significant effect on NDF, ADF and cellulose content which could be attributed to the fungal treatment in contrast to water socking of the TS in the present study. Furthermore, the same authors reported significant effect of duration of fermentation on composition of some nutrient and DM digestibility with a decreasing trend of fibers with increasing fermentation period, confirming further degradation. Their result had certain similarity with the result of the present study for which the OM and CP content and IVOMD of the FTS were higher at EP_{21} than EP_{14}, though they were lower at EP_{28}. The lower content of these nutrients at the higher EP and AR suggests that DM might have been lost (Kung, 2013) due to increased solubilization (Mulugeta, 2015). In support of this, Higa and Wididana (1991) stated that the action of the microorganisms in

the EM liberates various low-molecular weight organic compounds by decomposition of complex compound that can either be used up by the microbes (Widjaja et al 2016) or released as gaseous los.

5 CONCLUSION

From the results, it was concluded that cold water socked TS ensiled for 21 days with EM solution at a rate of 500mL EM /kg TS to have better nutritional quality than those ensiled for 14 and 28 days at rates of 250 and 750mL EM /kg TS application. As the pH of the silages was not satisfactorily lowered and color change was seen when the silage was exposed to air that may indicate feed out stability problem, future studies should focus on the EM application with addition of other additives that have fermentation stimulation and/or inhibition role.

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