Evaluation of Activated Effective Microorganisms (EM-2) as Biological Crop Residue Treatment Option Targeted for Feeding Crossbred Dairy Cattle

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Abstract

The study was conducted at Holetta Agricultural Research Center with the objective to evaluate the effect of ensiling crop residues (wheat, barley and oat straws) with activated effective micro-organism solution (EM2) on the chemical compositions, in-vitro digestibility and performances of mid lactating Boran-Fresian crossbred cows fed four dietary treatments. These were: ad libtum EM2 treated barley straw basal diet plus on-station formulated dairy concentrate mix supplemented @ 0.3 kg lt^{-1}(T1); 0.5 kg lt^{-1}(T2); 0.7 kg lt^{-1}(T3) and untreated barley straw basal diet plus 0.5 kg lt^{-1}milk yield (T4: control groups). Crude protein (CP), digestible organic matter in the dry matter (DOMD), estimated metabolizable energy (EME), total ash, neutral detergent fiber (NDF), acid detergent fiber (ADF) and lignin ($P<0.05$) were significantly ($P<0.05$) increased by EM2 treatment as compared to the untreated straws. Dry matter (DM) and organic matter (OM) losses as a result of EM-2 treatment were substantial ($P<0.05$) for all the three crop residues studied. Except for the ash content, interaction effects between the type of crop residue, rate of application and incubation durations were non-significant ($P>0.05$). Daily intake of EM2 treated barley straw was significantly higher ($P<0.05$) for all experimental cows compared to cows receiving the untreated residue. Similarly, daily total DM intake followed the same trend as for the basal feed intake. In general, daily intakes and apparent digestibility of all nutrients except DM & OM were higher ($P<0.05$) for cows fed the EM2 treated barley straw as a basal diet. Daily milk yield and compositions other than milk lactose and total solids were significantly different ($P<0.05$) among cows receiving the treated barley straw diet. On the other hand, due to high cost of straw treatment, compared to cows on the control diet, the gross and net profit obtained from intervention diets were marginal. In conclusion, EM2 can serve as an alternative biological treatment option for crop residues and thus, can be used on a wider scale among the livestock farming community to alleviate the inherent problems (low intake and digestibility) of most crop residues under local conditions in Ethiopia.

Key words: *Effective Microorganism, Activated EM (EM2), barley, wheat and oat straws*

Introduction

With a total production of about 50 million tonnes per annum (CSA, 2014) and estimated relative contribution exceeding 50%, the role of crop residues as a basal diet to ruminant livestock under Ethiopian context will continue to dominate the basal feed resource base even in the years to come. Despite their production potential and long history of utilization, the animal industry in

Ethiopia have not maximally benefited from this vast feed resources owning to their inherently poor nutritional value and associated negative effects on feed intake and digestibility. Thus, upgrading of straw quality should be a focal point of research strategy for improving ruminant livestock production in the country. In this regard, during the last two to three decades both scientists and extension workers have shown great interest in chemical and physical treatment of straw (Sundstol and Owen, 1984). The ammoniation method using urea has received major attention as an appropriate system in the developing countries (Owen and Jayasuriya, 1989a). However, the success with regard to on-farm application of ammonia treatment as well as other chemical methods has generally been disappointing. Consequently, many attempts have been made by scientists to find other efficient approaches to address the problems. A promising alternative to chemical treatment is a microbial fermentation method. This method is simple in application and is of low cost, and the farmer can use the same urea-ammonia treatment facilities to carry out the process.

The technology of Effective Microorganisms (EM) as biological inoculants was developed in the 1970's at the University of the Ryukyus, Okinawa, Japan. The inception of the technology was based on blending a multitude of microbes, and was subsequently refined to include three principal types of organisms commonly found in all ecosystems, namely Lactic Acid Bacteria, Yeast Actinomycets and Photosynthetic bacteria (Higa, 1996). A variety of dry crop residues have been successfully ensiled with addition of microorganisms. Being organic in nature, microbial ensilage of crop residues increases daily gains, feed intake and feed conversion, and decreases feed cost per unit gain in growing ruminants (Zhang and Meng, 1995; Ma and Zhu, 1997; Konoplya and Higa, 2000; Hanekon*et al*., 2001).

Although the possibility of biological method of straw treatment has a great appeal as an alternative to the use of expensive (in terms of money and energy) chemicals and environmental pollution, many aspects need further investigation under local conditions. The objective of this study was to evaluate activated microbial inoculant EM2 solution as a technologically and biologically feasible alternative crop residue treatment and feeding options for dairy cattle in Ethiopia.

Materials and Methods

Description of study area

The study was carried out on-station at Holeta Agricultural Research Center. The center is located about 35 km West of Addis Ababa along the main road to Ambo. The study area has an altitude of 2400 meters above sea level (m.a.s.l) and receives an average annual rainfall of about 1055 mm. The mean minimum and maximum temperatures are 6.1^{o}C and 22.2^{o}C, respectively.

Experimental feed preparation and designing treatment protocols

This laboratory trial has focused on ensiling three cereal crop residues, i.e., wheat, barley and oat straw with EM2 (extended EM solution). EM2 was prepared according to the procedure of

EMROSA (2004) by mixing EM1 with molasses and chlorine free water in the ratio of 1:1:18 respectively. 10% molasses was added to the solution to provide nutrients specifically sufficient soluble carbohydrates to the microbes in the EM2 solution and thereby to facilitate the ensiling process. EM2 solution was then applied to the residues at the rate of 0, 1 and 1.5 lt, kg^{-1} DM of the residues. Except for the untreated crop residue the materials were then incubated for 30 and 40 days using airtight plastic containers. Straws of wheat, barley and oat samples from known varieties were collected from on-station plots and subjected to chopping to an approximate size of 3-5cm. At the end of the incubation period part of the silage mass was subjected to oven drying at 65^{o}C for about 72hours for partial DM determinations and further processing to 1-mm sieve size grinding for laboratory chemical compositions and *in-vitro* OM digestibility studies.

Experimental animal selection and management

A total of four lactating F1 crossbred cows (Boran x Friesian) were used for this experiment. Experimental cows with similar lactation performance (8-10 lt,d^{-1}), same stage of lactation (mid-lactating i.e., three months after calving), and body weight of 393±25kg but differing in parities (two through five) were selected from the total dairy herd available on station. All the cows were weighed and drenched with broad-spectrum anti-helminthics (Albendazole 500mg) prior to the start of the experiment. The cows were individually stall-fed in a well-ventilated barn with concrete floor and appropriate drainage slope and gutters.

Experimental design, treatments and measurements

At the beginning of the experiment, four cows were randomly blocked in a simple 4X4 Latin Square Design. There were, in general, 4 experimental cows, 4 treatment diets and 4 periods. The length of each period was 28 days, out of which 21 days were allocated for adaptation while the remaining seven days were used for actual data collections and analysis. In total, the feeding trial has taken about 112 days. All cows were hand- milked twice a day and milk yield was recorded daily. Aliquot samples of morning and evening milk was collected weekly to analyze milk chemical composition. Water was available at all times free of choice. The experimental animals were randomly receiving one of the four dietary treatments indicated below.

1. EM2 treated barley straw basal diet *adlibitum* + 0.3kg concentrate mix, lt^{-1} of milk produced
2. EM2 treated barley straw basal diet *ad libitum* + 0.5kg concentrate mix, lt^{-1} of milk produced
3. EM2 treated barley straw basal diet *ad libitum* + 0.7kg concentrate mix, lt^{-1} of milk produced
4. Untreated barley straw *ad libitum* + 0.5 kg concentrate mix, lt^{-1} of milk produced (control diet)

The cows were offered the supplements twice a day with a standard on-station formulated dairy concentrate mixture (76% wheat bran, 23% noug seed cake and 1% salt). The mix was assumed

to fully meet the daily requirement for protein (16%) in the total ration of lactating crossbred cows with milk yield of 8-10 lt, d^{-1} and a butter fat content of 4.5% as described in ARC (1990) when fed as supplement at the rate of 0.5 kg/liter of milk. Barley straw collected from Holetta Agricultural Research Center was harvested by combine harvester, immediately baled and stored in hay shed until it was ready to be chopped to a size of 3-5cm using electrical chopper. The process of ensilage begins with spraying of EM2 solution to the barley straw at the rate of 1lt per kg straw mass. The treated barley straw was compacted and then allowed to ferment for one month in an air tight plastic barrel of (250 lt) capacity before it was being fed to the animals.

Feed offer and refusals were measured and recorded for each cow to determine daily feed and nutrient intake. Feed offer and refusal samples were taken daily and weighed per cow, bulked on a period bases and oven dried at 65^0C for 72h. Samples were then ground using Cyclo-Tec sample mills to pass 1 mm sieve size for DM analysis to calculate feed intake.

Diet apparent digestibility

Apparent digestibility was determined for the total ration in each treatment using the procedures of total fecal collection method for a period of five consecutive days at around the end of each experimental period. To minimize error in faces collections, farm personnel were assigned around the clock to scoop feces into plastic buckets when the animals were defecating. Urinal contamination was minimized by frequent washing of the concrete floor with high pressure running water using a plastic water hose. Individual cow's feces were weighed every morning before 8:00am and before feeds were given to the animals. The feces from each cow were thoroughly mixed and a sample of 1% were taken and placed in polyethylene bag. Composite samples of the daily collected samples were mixed and stored in a deep freezer (-20^{o}C) until the end of the collection period. At the end of the collection period, the pooled samples were thawed and mixed thoroughly and samples were oven dried at 65^{o}C for 72 hours, ground to pass a 1-mm sieve and stored in sample bottles at room temperature. Apparent digestibility of DM and nutrients was determined using the formula:

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Milk yield and composition

The cows were hand- milked twice a day at 5:00am in the morning and 16:00pm in the afternoon and milk yield was recorded individually for each animal. 100ml of milk Aliquot samples from the morning and evening milking were taken at each period on a weekly basis for laboratory determination of major milk components (milk fat, protein, lactose and total solids). The sampling bottle was properly cleaned and sanitized before samples were taken to Holetta Agricultural Research Center dairy laboratory.

Chemical analysis

All samples of feeds from laboratory trial in phase one, feed offer and refusals samples from the feeding trial in phase two and feces samples from digestibility trial were analyzed for DM, ash, N (Kjeldahl-N) according to the procedures of AOAC (1990). Neutral detergent fiber (NDF), Acid Detergent fiber (ADF) and permanganate lignin were determined by the method of Van Soest and Robertson (1985). *In-vitro* OM digestibility of feeds offered was determined according to the procedures outlined by Tilley and Terry (1963). Hemi-cellulose was calculated as a difference between NDF and ADF. Metabolizable energy (ME) value was estimated from the *in-vitro*OM digestibility (IVOMD): EME (MJ/kg) =0.16(IVOMD) according to McDonald *et al*. (2002). Gerber method (AOAC, 1980) was used for milk fat analysis, while the formaldehyde titration method (Pyne, 1932) was used to analyze milk protein. Total solids in the milk were determined using the procedures described by Richardson (1985). Lacto scope milk product analyzer (Users manual ver. 1.1., 2000) was used for lactose determination.

Cost-benefit analysis

Economic returns were calculated for the different groups of animals based on current price data collected for each input and out price from local markets around Holetta town. A partial budget analysis has been employed to analyze those items of income and expenses that change. Therefore, the costs of EM2 treatment per kg straw mass, concentrate feed ingredients and the cost for treated barely straw consumed by the animals in the different treatment group were considered as varying costs while all other costs (wedge, medications, electricity, water etc.) were ignored since they remained constant over all the dietary treatments.

Statistical analysis

Analysis of variance was made using a statistical package SAS (SAS, 2002). Data from the first laboratory trial was analyzed using CRD model in 3x3x2 factorial arrangements. All data from the feeding and digestibility trial was analyzed using a simple 4X4 Latin Square Design. Treatment means were separated using Least Squares Significant difference (LSD). The models for both designs are indicated below:

1. Model for CRD in factorial arrangement
   $Y_{ijk} = \bullet + C_i + L_j + CL_{ij} + e_{ijk}$Where; $\bullet$ = Overall mean, C_i= Effect of type of crop residue,L_j =Effect of level of application of EM2,CLij = Interaction effect,e_{ijk} =Random error
2. Model for simple 4X4 Latin Square Design
   $Y_{ijk} = \bullet + C_i + P_j + T_k + E_{ijk}$,Where: $\bullet$ = Overall mean,C_i = Cow effect (parity), P_j = Period effect,T_k = Treatment effect,E_{ijk} = Experimental error

Results and Discussion

Chemical compositions and In-vitro digestibility of EM-2 treated cereal residues

Responses of major cereal residues to EM-2 ensiling are presented in Table 1. There was significant increment ($P<0.05$) in the total ash and CP, NDF, ADF, lignin and DOMD contents of major cereal residues ensiled with EM2. For ash, this amounts to 20.8%, 22.8% and 19.2% for oat, barley and wheat straw, respectively over their untreated counterparts. The increment for CP was 14.6% for oat, 14.2% for barley and 25.5% for wheat over the untreated residues. Similarly, percentage DOMD increments over untreated residues were 19.5%, 26.0% and 39.5%, respectively, for oat, barley and wheat straw. Hemicelluloses content had increased by 13.6%, 27.1% and 44.7% over the untreated residues of oat, barley and wheat, respectively. On the other hand, when EM2 was used as biological inoculants there was significant ($P<0.05$) reductions in the OM and improvements in the cell wall (NDF, ADF and lignin) constituents over the untreated residues. The reduction of OM for oat, barley and wheat was 2.0%, 1.9% and 1.6%, respectively. The percentage improvement in NDF contents of the residues were 4.8%, 5.6% and 6.1% for oat, barley and wheat straw, respectively while EM has improved the remaining cell wall constituents of oat, barley and wheat straw in that order by 9.6%, 13.5% & 20.0% for ADF; and 9.30%, 25.2% and 19.6% for lignin.

In general, all except OM constituent in the residues were positively influenced by EM treatment. However, responses of the residues to change in ash, CP, DOMD and cell wall constituents because of EM2 ensiling were quite appreciable. Among the treated residues the response of wheat straw followed by barley straw to EM treatment was much higher supporting previous notions that poor quality residues will always respond much better than residues with relatively better nutritional qualities. The reduction in OM contents of EM2 ensiled crop residue from the current trial is also in agreement with previous report by EL-Tahan (2003) for fungal treated and untreated wheat straw. Salman *et al.* (2011) held an experiment that aimed to evaluate the effect of biological treatment with fungi, yeast and bacteria or their combinations on the nutritive value of sugar cane bagasse (SCB) and found a decreased DM for treated residues while the ash was observed to have been significantly increased. Under local condition, increased ash contents and hence decreased organic matter contents have also been observed by Yonatan *et al.* (2014) for coffee pulp treated with EM solution. The increment in ash contents for EM treated residues can be linked to the presence of molasses feed ingredient reportedly high in some minerals. Reduction in the OM contents in the present trial can be liked to microbial solubilizing and fermentation of organic materials (mainly structural carbohydrates) as energy sources for their own growth and multiplications as indicated by El-Ashry*et al. (*2003) and Rolz*et al*. (1988)

Table1. Response of major cereal residues to EM2 ensiling

Treatment	DM (%)	Average nutritive value expressed as % DM						
		Ash	CP	NDF	ADF	H-cell	Lignin	DOMD
UOS	93.1^{b}	8.81^{c}	1.92^{e}	80.7^{e}	63.9^{d}	16.8^{c}	9.68^{b}	38.89^{d}
UBS	93.5^{a}	7.59^{d}	2.74^{b}	79.6^{d}	64.1^{d}	15.4^{d}	10.9^{bc}	38.94^{d}
UWS	93.8^{a}	7.70^{d}	1.65^{f}	83.0^{f}	65.2^{e}	17.8^{bc}	11.9^{c}	29.64^{e}
TOS	91.4^{d}	10.6^{a}	2.20^{c}	76.8^{b}	57.8^{c}	19.0^{b}	8.8^{ab}	46.46^{b}
TBS	92.9^{b}	9.32^{b}	3.13^{a}	75.1^{a}	55.5^{b}	19.6^{b}	8.18^{a}	48.67^{a}
TWS	92.2^{c}	9.18^{bc}	2.07^{d}	77.9^{c}	52.2^{a}	25.7^{a}	9.55^{b}	41.36^{c}
Mean	92.8	8.87	2.29	78.9±1	59.8±	19.1	9.84	40.66
±SEM	±0.32	±0.42	±0.20	.05	2.01	±1.34	±0.51	±2.50
CV%	1.07	7.08	1.56	2.17	10.12	14.82	7.26	6.16

^{abc} *Means with different superscripts along column are significantly different (P=0.05); UOS= untreated oat straw; UBS= untreated barley straw; UWS= untreated wheat straw; TOS= treated oat straw; TBS= treated barley straw; TWS= treated wheat straw; DM=dry matter; OM= organic matter; CP= crude protein; NDF=neutral detergent fiber; ADF=acid detergent fiber; H-cell=hemi-cellulose; DOMD =digestible organic matter in the dry matter*

The average CP improvement over the untreated residues (i.e., 17%) from the current trial can fairly be compared with previous research findings of 19.2% for various microbial treated fibrous basal diets by Nahla *et al*. (2015) and El-Marakby (2003). Improvements in CP contents of EM2 treated residues may be due to one of the following reasons: the presence of microorganisms, extracellular enzymes and residual media ingredients in the treated materials (Khattab*et al.*,2013), the capture of access nitrogen by aerobic fermentation by fungus (Akinfemi, 2010), and the proliferation of fungi during degradation (Akinfemi and Ogunwole, 2012).The increments in CP contents due to EM treatment, however, were so much marginal compared to progress made with biological treatments earlier for other fibrous diets (El-Banna*et al.*, 2010b;Akinfemi and Ogunwole, 2012).

The observed increment in *in-vitro* OM digestibility of the residues ensiled with EM could be attributed to the improvements in major cell wall constituents (NDF, ADF and lignin). The yeasts and bacterial species present in the EM might have positively induced the change that was reflected by improvement in the corresponding *in-vitro* DM digestibility values of the treated residues. Especially the role of yeast in the EM solution is quite indispensable since yeasts have been reported to utilize feeds with high structural components (Maurya, 1993). The maximum improvement in DOMD brought about by EM2 treatment over untreated residue from the current trial was the one that was recorded for wheat straw (39.5%). The average improvement over the untreated residue (i.e., 28%) was close to the figure (30%) reported earlier for EM ensiled coffee pulp by Yonatan (2014). IVOMD figure as high as 57.02% was reported for rice straw treated with different strains of fungi earlier by Akinfemi and Ogunwole (2012).

Application of EM inoculates on fibrous feedstuffs have been previously reported to have increased the quality of the silage by decreasing fibrous contents of the silage (NDF and ADF) (Higa and Wididana, 2007). Possible rationale behind a reduction in NDF and ADF content of the ensiled residues in the current trial according to Fayed *et al.(*2009) could be due to the addition of molasses to the silage which in effect can increase the number of anaerobic bacteria (lactic acid bacteria: *Lactobacillus plantarum; L.casei; Streptococcus lactis) and* yeast (Cercomycaecervicae) capable of degrading the lingo-cellulotic complexes in the cell wall fractions of the silage material through their oxidizing and solublizing effects. The current result is also in pare with the findings of El-Marakby (2003) who found a great decrease in content of neutral detergent fiber (NDF- 45.1%), acid detergent fiber (ADF- by 31.5%), cellulose (by 53.7%) and hemi-cellulose (by 96.3%) for wheat straw treated with white rot fungus, *Agaricusbisporous.* All disparities with previous findings can be speculated to the difference in the type of microbes and/or microbial strains used, quantities applied, straw type and quality and above all luck of reconstituting the residues with water prior to EM applications.

Response of crop residues to levels of EM2 applications and durations of incubations

Responses of major cereal residues to quantities in volumes of EM2 applied per kg straw mass and days required to come up with best quality straw silage as measured through chemical compositions and *in-vitro* OM digestibility is shown in Table 2. Except for the ash content, the level of application of EM2 solution per kg straw mass was non-significant (P>0.05) for all other nutritional parameters under consideration. Similarly, regardless of the difference in the ensiling periods, there were no detectable changes (P>0.05) in both chemical compositions and in-vitro digestibility coefficients except for OM of the residues incubated for 30 and 40 days. In other words, there were no net gains in nutritional values by adding extra ten days beyond 30 days of incubations. Interactional effects between straws, rates of EM2 applications and incubation periods for all laboratory quality parameters considered in this particular studies were very weak and happen to remain non-significant (P>0.05).

The fact that interactional effects were non-significant has led to the decision to consider the three independent factors for the different quality parameters considered. Accordingly, the absence of statically detectable nutritional quality differences (P>0.05) for EM2 application rates can lead to the further recommendation of EM-2 @ 1lt, kg^{-1} dry straw mass for use on a wider scale at an on-farm level. The nutritional quality of the residues treated with EM2 and subjected to incubation at two different ensiling periods (30 and 40 days) did not happen to show any statistically (P>0.05) appreciable differences. Thus considering both factors 1lt EM2, kg^{-1} dry residue weight incubated for a period of 30 days can be recommended for on-farm applications under the present conditions of smallholder dairy farmers in the central highlands of Ethiopia.

Table 2. Responses of crop residues to rates of EM2 applications and durations of incubations

Variables	DM %	Average nutritive value (% DM)						
		Ash	CP	NDF	ADF	H-cell.	Lignin	DOMD
1 lt EM2/kg DM	92.58[a]	9.45[b]	2.49[a]	76.48[a]	56.68[a]	19.80[a]	8.77[a]	46.23[a]
1.5 lt EM2/kg DM	92.17[b]	9.97[a]	2.44[a]	76.74[a]	56.97[a]	19.78[a]	8.90[a]	45.76[a]
Mean ± SEM	92.38 ±0.01	9.71 ±0.01	2.47 ±0.01	76.61 ±0.01	56.83 ±0.03	19.79 ±0.01	8.84 ±0.05	45.50 ±0.13
30 days of ensiling	92.04	9.85	2.45	76.72	56.70	20.01	8.87	45.50
40 days of ensiling	91.71[b]	10.23[a]	2.49[a]	76.50[a]	56.94[a]	19.57[a]	8.69[a]	45.49[a]
Mean ± SEM	91.88 ±0.30	9.72 ±0.09	2.47 ±0.01	76.61 ±0.02	56.82 ±0.02	19.79 ±0.04	8.78 ±0.29	45.50 ±0.01
Straw X EM2 X Incubation	0.103	0.106	0.073	0.917	0.231	0.554	0.138	0.061

[abc] Means with different superscripts along a column are significantly different (P=0.05); DM=dry matter; OM= organic matter; CP= crude protein; NDF=neutral detergent fiber; ADF=acid detergent fiber; H-cell=hemi-cellulose; DOMD =digestible organic matter in the dry matter

Chemical compositions of experimental feed ingredients

The chemical compositions of feeds used for feeding trial in the present study are shown in Table 3. Higher CP contents were observed for the concentrate mix. There was also improvement in CP contents in EM-2 treated straw as compared to the untreated straw. The untreated barley straw used in this study contained 27.6%, 31.7%, 15.6% and 27.6% more NDF, ADF, Hemi-Cellulose and Lignin content on DM basis than the treated barely straw, respectively. In this regard, Samsudin, *et al.* (2013) was also able to note significant differences among the EM treated rice straw and untreated rice straw in DM, OM, CP, NDF, ADF and cellulose contents.

Table 3.Chemical compositions and *in-vitro* digestibility of experimental feed ingredients (% DM basis)

Feed type	DM (%)	OM	CP	DOMD	EME (MJ/kg DM)	NDF	ADF	HC	Lignin
EMTBS	90.09	89.93	4.95	51.7	8.27	57.97	40.66	17.31	8.03
UTBS	93.4	92.39	2.30	33.1	5.29	80.05	59.56	20.49	11.05
Concentrate	89.0	92.10	20.0	68.0	10.88	40.00	21.30	18.70	6.51

EMTBS = EM treated barley straw; UTBS=untreated barely straw; HC=hemicellulose; OM= organic matter; CP= crude protein; ADF=acid detergent fiber; DM=Dry matter; NDF=Neutral detergent fiber; MJ=Mega joule; IVOMD =Invitro organic matter digestibility; EME= Estimated metabolizable energy

The level of DOMD and EME contents observed for the treated barley straw was much higher than that observed for the untreated barely straw. However, the values were much lower compared to that observed for the concentrate mix used in the study. Akinfemi and Ogunwole

(2012) also reported higher EME for the fungal treated rice straw than the untreated residue. On the other hand, treatment with EM2 has almost doubled the CP contents over the untreated residue. The improvement made in cell wall fraction over the untreated residue of barley straw was also remarkably higher. Since intake and digestibility limitation with untreated residue can somehow be improved with EM treatment (Table 4&5) it is natural to expect additional saving from daily concentrate allowance of lactating crossbred cows maintained on EM-2 treated crop residue based diet.

Daily feed and nutrients intake

The values for voluntarily feed and nutrient intakes of experimental cows are presented in Table 4. There were considerable changes ($P<0.05$) in the daily basal feed intakes between the groups that fed with the treated and untreated barley straw residues. Difference in the daily allowance of concentrate were non-significant ($P>0.05$) for cows under dietary treatments receiving the treated barley straw. Experimental cows receiving the treated barely straw as a basal diet consumed on average 6.62 kg,d^{-1} while those on the untreated residue consumed 1.76 kg less barley straw on a daily basis. Daily allowance for concentrate and total dry matter intakes were significantly differing ($P<0.05$) both among the groups that were receiving the treated residues and when these same groups were compared with cows receiving the control diet.

Daily Nutrient intakes followed same trend as for the total DM intake. In general, DM intake differences were significant ($P<0.05$) both among and between dietary treatments with cows on dietary T3, consuming considerably higher daily nutrient intakes followed by cows on dietary T2 and T1. Except for ADF intakes the increasing trend for all nutrient intakes followed the increasing trend in the daily allowance of concentrate intakes among cows receiving the treated residue. Because of the response of ADF residue to EM2 treatment was so marginal (Table 3), average daily intake of ADF fraction by cows receiving the untreated residue as a basal diet was higher by 0.13kg than those cows receiving the treated barely straw residue. Metabolizable energy (MJ,d^{-1}) intake differences were highly significant among all dietary treatments ($P <0.05$) with cows on dietary treatment 3 consuming considerably higher daily ME per day of 15.07, 30.08 and 47.04 compared to that of cows in T2 ,T1 and T4, respectively. Cows on all treatments were on the negative energy balance for the targeted daily milk yield of 8-10 kg according to ARC (1990) presumably because the total ration was not fortified with adequate energy sources both quantitatively and qualitatively taking the quality of the basal diet in to account.

Using wheat straw and other different crop residues that are microbially treated and fed to different class of animals in China, Meng*et al.* (1999) reported similar improvements in the daily basal and nutrient intakes for DM, OM, CP, NDF and ADF. These changes according to same authors were related to the fact that ensiled crop residues with microbial agents usually have good palatability for ruminants, and thus would be responsible for higher intake. More over according to Yoseph*et al.* (2002) lower fiber and relatively higher CP contents in the treated residue may be responsible for the improved DM and total DM intakes by ruminants. On the

contrary, negative responses in feed and nutrient intakes have also been reported by El-Banna*etal. (*2010a) and Abd El-Galil(2011) for biologically treated crop residue based diets for various classes of animals compared to the untreated residues. These variations can be speculated to the difference in the microbial agents used; type of residues subjected to the biological treatment and the difference in the experimental animal unit and/or the environments under which the specific trials were conducted.

Table 4. Dry matter and nutrient intake (kg/d/cow) of lactating crossbred dairy cows

Intake	Treatments				
	T1	T2	T3	T4	SEM
Barely straw	6.65^{a}	6.68^{a}	6.54^{a}	4.86^{b}	0.17
Concentrate	1.72^{c}	3.05^{b}	4.48^{a}	2.84^{b}	0.34
Total DM	8.37^{c}	9.73^{b}	11.02^{a}	7.65^{c}	0.34
Total OM	7.57^{c}	8.80^{b}	9.99^{a}	7.06^{c}	0.31
CP	0.68^{c}	0.94^{b}	1.22^{a}	0.68^{c}	0.07
NDF	4.58^{c}	5.14^{b}	5.67^{a}	5.00^{bc}	0.16
ADF	3.02^{b}	3.28^{ab}	3.49^{a}	3.39^{a}	0.09
ME (MJ/day)	74.72^{c}	89.73^{b}	104.8^{a}	57.76^{d}	3.80

^{abc} Means with different superscripts within a row are significantly different (P<0.05); SEM=standard error of mean; DM = Dry matter; CP = Crude protein; NDF= neutral detergent fiber; ADF acid detergent fiber; ME = Metabolizable energy; T1=EM2 treated barley straw basal diet adlibitum + 0.3kg concentrate mix/liter of milk produced; T2=EM2 treated barley straw basal diet ad libitum+ 0.5kg concentrate mix/liter of milk produced; T3=EM2 treated barley straw basal diet ad libitum+ 0.7kg concentrate mix/liter of milk produced; T4=Untreated barley straw ad libitum + 0.5 kg concentrate mix/liter of milk produced (control diet)

Apparent digestibility of dry matter and major nutrients

The results of the effect of EM2 treated barely straw supplemented with concentrate mix on total diet apparent nutrient digestibility of lactating cross breed dairy cows are presented in Table 5. Total diet apparent nutrient digestibility appeared to be significant (P<0.05) over experimental cows that were maintained on the control diet except for DM and OM. Accordingly, cows fed with the treated barley straw as basal diet digested on average 11.89%, 9.52% & 7.57% more CP, NDF and ADF, respectively, over the cows receiving the control diet. Among cows in the intervention group, however, more nutrients except DM and OM were digested by cows receiving dietary T3. Compared to the control group cows on dietary T3 effectively digested more CP, NDF and ADF calculated to be greater by 18.6, 13.6 and 10.57 percentage units, respectively.

In general, it can be said that the improvements in apparent nutrient digestibility have been clearly reflected by a more and progressive daily intakes for cows that have been receiving the treated barley straw residue (Table 4). The effect of dietary treatment was more remarkable for cows receiving diet-1 (T1) in light of the fact that these cows consumed less concentrate (<200 g,d^{-1}), as were managed to eat more basal feed compared to cows on the control group. A

tendency for the increased apparent digestibility for all nutrients among cows fed with EM2 treated barely straw compared to the control group may be explained by the higher degradability rates of the treated barley straw in the rumen owning to the delignification process during the ensiling process which renders more cellulose and hemi-cellulose for microbial colonization and fermentations in the rumen. It could also be related to higher dietary total DM intake among the treated residues compared to the control group (see Table 4 above).

The result of the current finding is also in agreement with El-Banna*et al.*(2010a) who reported that the digestibility coefficients of DM, OM, CP, NDF, ADF, hemi-cellulose and cellulose of *Lactobacillus acidophilus and* brown rot fungi *Trichodermareesei F-418* treated potato vines and sugar cane bagasse (SCB) were higher than those of untreated potato vines and SCB. Guim*etal. (*2000) further stated that DM digestibility percentage of EM treated silage resulted to significant levels of increment in the digestibility of CP than untreated silage. The higher digestibility percentage of CP for cows under T3 can be justified by the higher intake of concentrate mix and hence of CP intake (see Table 4 above) compared to cows on the remaining treatments.

Data analysis from the current trial showed that, for cows receiving the intervention diet the cell wall digestibility was significantly increased (P<0.05) over the untreated residues. The finding is in agreement with earlier report by by Abd-Allah (2007) for a biologically treated Vs untreated corn cobs. The improvement in cell wall digestibility coefficients as a result of biological treatments according to Nsereko *et al.* (2002) may be due to the effect of increasing numbers of cellulolytic bacteria and fungi in the rumen, responsible for the stepwise hydrolysis of cellulose to glucose.

Table 5. Feed DM and nutrient apparent digestibility of experimental cows

Apparent digestibility (%)	Treatments				SEM
	T1	T2	T3	T4	
Dry matter	47.65	51.17	52.57	39.91	4.19
Organic matter	51.09	54.51	55.92	45.01	3.89
Crude protein	50.01^{b}	55.87^{a}	63.01^{a}	44.412^{c}	4.44
Neutral detergent fiber	43.93^{b}	45.32^{b}	50.38^{a}	37.02^{c}	4.2
Acid detergent fiber	34.62^{b}	38.33^{a}	40.98^{a}	30.41^{c}	4.38

^{abc} Means with different superscripts within row are significantly different (P<0.05); T1=EM2 treated barley straw basal diet adlibitum + 0.3kg concentrate mix /liter of milk produced; T2=EM2 treated barley straw basal diet ad libitum+ 0.5kg concentrate mix /liter of milk produced; T3=EM2 treated barley straw basal diet ad libitum+ 0.7kg concentrate mix /liter of milk produced; T4=Untreated barley straw ad libitum + 0.5 kg concentrate mix /liter of milk produced (control diet)

Milk yield and compositions

Results of the effect of dietary treatments on mean daily milk yield and compositions are presented in Table 6. There were significant differences (P < 0.05) in milk yield among treatments. Cows that were maintained on diet 3 (T3) produced extra daily milk of 0.55, 0.65 and

1.07 kg over those cows that were maintained on the remaining dietary treatments. The extra daily milk produced by these cows might not only be associated to the improved basal feed intake but can also be justified by the relatively larger daily concentrate intake and hence, protein and energy intakes than cows on the other treatments. Cows receiving T1 produced significantly (P<0.05) more daily milk yield (0.42 kg, d^{-1}) over the cows receiving the control diet and the same amount of daily milk yield (P>0.05) as cows on dietary T2. When the efficiency of milk production is compared taking in to account the daily concentrate allowance, cows which were receiving T1, T2, T3, and T4 consumed 0.267kg, 0.466kg, 0.632kg and 0.472kg, respectively for each kg of milk production. This implies that cows under T1 were efficient and more economical since less concentrate (0.267 g, d^{-1}) was consumed to produce a kg of milk.

Similar to the current findings, Nahla*et al*. (2014) indicated that lactating cows fed diets based on microbial ensiled straw had increased milk and fat-corrected milk yield, and slightly higher milk fat percentages compared with diet of untreated straw. Some other researchers (Moawd, 2003; Khattab, *et al.* 2011) who have also used biologically treated wheat straw and/or rumen contents to either lactating sheep or goats reported same findings that agree with the finding of the current trial for milk yield and compositions compared to that recorded for the untreated residues.

Table 6.Milk yield (kg/d) and compositions (%) of lactating crossbred cows

	Treatments				
Variables	T1	T2	T3	T4	SEM
Daily milk yield	6.440^{b}	6.540^{b}	7.09^{a}	6.02^{c}	0.18
Fat	3.85^{b}	3.92^{ab}	4.04^{a}	3.71^{c}	0.065
Protein	2.97^{ab}	2.98^{a}	3.09^{a}	2.91^{b}	0.05
Lactose	5.00	4.76	4.91	4.88	0.14
Total solids	12.41	12.40	12.45	12.43	0.10

^{abc} Means with different superscripts within row are significantly different at (P<0.05); T1=EM2 treated barley straw basal diet adlibitum + 0.3kg concentrate mix /liter of milk produced; T2=EM2 treated barley straw basal diet ad libitum+ 0.5kg concentrate mix /liter of milk produced; T3=EM2 treated barley straw basal diet ad libitum+ 0.7kg concentrate mix /liter of milk produced; T4=Untreated barley straw ad libitum + 0.5 kg concentrate mix /liter of milk produced (control diet)

Cows fed with the EM treated barely straw produced higher milk fat content (P<0.05) than cows in the control group. The higher fat percentage (P<0.05) by cows on T3 over cows receiving dietary T1 and T4 could be related to higher total DM, nutrient intake and digestibility (see Table 4 and 5). In line to this, Kholif*et al.* (2014) reported increased fat contents for *Pleurotusostreatus* treated rice straw fed lactating Baladi goats (38 and 40 *vs*. 34 g h^{-1},d^{-1}) compared with those fed untreated rice straw. The improvement in fat contents of the milk produced from lactating animals fed with feeds treated with biological agents, according to these researchers, was perhaps linked to the increased levels of milk conjugated linoleic and unsaturated fatty acids obtained from the increased daily intake of the treated barley straw. Milk

protein percentages also varied significantly (P<0.05) with cows receiving T2 and T3 having the highest protein percentage unit over those cows that were receiving the control diet. Increased dietary CP intake from the daily concentrate allowance (see Table 4) might have helped cows in these groups generate the observed difference in milk protein. Phipps (1994) attributed higher daily milk yield and protein concentration to higher daily protein intakes of lactating cows. On the other hand, no considerable differences (P>0.05) observed for cows that were receiving EM treated barley straw as intervention basal diet and when these similar groups were compared with the control group for milk lactose and total solids. It is unclear why milk sugar (lactose) was not affected by different dietary treatments despite marked differences in the daily concentrate allowance of the cows existing under the different dietary treatments. It is also hardly possible to explain the absence of significant difference among all dietary treatment for milk total solids while still considerable improvements were made to other compositional parameters except for milk lactose. It should be noted that, negative responses in daily milk yield and compositions have also been reported elsewhere by Kholif*et al. (*2014) and Milenković*et al. (*2004).

Economic return obtained from EM2-treated barely straw feeding

Cost benefit analysis indicated that experimental cows receiving the control diet were better in terms of the daily gross return on the individual animal basis. This gross return when calculated over cows maintained on the remaining dietary treatments was greater by 31.83, 35.83 and 33.95 Birr/d than those cows maintained over T1, T2 and T3, respectively.

Table 7. Economic return/cow/day of experimental cows fed different dietary treatments

Cost variables	T1	T2	T3	T4
EM-UBS	-	-	-	10.11
EM-TBS	58.39	58.65	57.42	-
Concentrate	6.15	10.94	16.07	10.19
Total variable cost	64.54	69.59	73.49	20.30
Income variables				
Milk sale	67.62	68.67	74.45	63.21
Dung cake sale	24	24	24	16
Total income	91.62	92.67	98.45	79.21
Gross return	27.08	23.08	24.96	58.91
Net return /control diet	-31.83	-35.83	-33.95	

UBS: untreated barley straw; TBS: Treated barley straw; T1=EM2 treated barley straw basal diet adlibitum + 0.3kg concentrate mix /liter of milk produced; T2=EM2 treated barley straw basal diet ad libitum+ 0.5kg concentrate mix /liter of milk produced; T3=EM2 treated barley straw basal diet ad libitum+ 0.7kg concentrate mix /liter of milk produced; T4=Untreated barley straw ad libitum + 0.5 kg concentrate mix /liter of milk produced (control diet)

Cows on dietary T1, however, generated more gross and net return over the remaining cows other than those on the control diet. More economic return by control cows can be justified to the rising cost of straw treatment with EM2 than it was originally anticipated. Moreover, the difference in the daily basal feed intake and the resulting produce in the daily milk of cows receiving the intervention diet were not large enough to offset the costs for straw treatment compared to cows in the control group. On the other hand, the relatively higher gross and net return per cow per day of cows in T1 group compared to same cows receiving treated straw based diet in T2 and T3 might have something to do with the reduction in the daily allowance of concentrate feed by 0.2 and 0.4 kg,d^{-1} over same treatments, respectively. In addition to the economic returns, biological responses to EM based diet would need to be judged by their long-term positive impact on general body conditions and reproduction responses of lactating dairy cows. Furthermore, considering the present cost of straw treatment with EM and the market price of milk, feeding EM treated straw would be economically much attractive if cows with higher milk production potential in early lactations are fed with EM treated straws of relatively poorer quality and cheaper price.

Assumptions

- Estimated labor cost per day was 70 Birr
- Cost of 1kg treated barley straw was 8.78 Birr
- An average fecal dry matter output of 4.01kg & 5.04kg for the control and cows on the intervention diets. With that assumption a cow on the control diet produced around 8 dung cakes/day while cows on the intervention diet produced around 12 dung cakes on same date.
- Sale price for a dung cake was 2 Birr while it was 10 Birr for a liter of milk
- Current exchange rate of Ethiopian Birr for 1 US dollar = 22.85

Conclusion

Nutritive value, intake and digestibility of cereal residues were considerably improved when a liter of EM2 solution was applied against a kg of crop residues on DM basis. Moreover, daily milk production response among the cows fed with EM2 treated barley straw based diet was substantially improved when the cows were supplemented with a dairy concentrate amounting to and/or above 0.3 kg,lt^{-1},d^{-1}. Future research work shall focus on minimizing cost of straw treatment mainly through reconstituting the residues with water prior to EM treatment. That way, the amount and cost of EM2 used kg^{-1} straw mass can be drastically reduced. The cost of treatment and hence of feeding can further be cut to a significant level if the initial purchase price of the preferred residue for EM2 treatment and ensiling is relatively cheaper. So under local condition, it could be more worthy to consider wheat straw than barley and teff straws.

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