

Urea-molasses Pre-treatment to Enhance Nitrogen Gain, Digestibility, Intake and Milk Yield from Crop-Residues in Smallholder Dairy Farms in Eastern Africa

Ongadi Patrick Mudavadi^{1,2*}, Mpolya Abraham Emmanuel¹,
Lukuyu Adubwa Bernard³, Haule Alphonse⁴, David Peter Ngunga⁵,
Gachuri Charles⁶, Muyekho Francis Namasake⁷
and Endalkachew Wolde-meskel⁸

¹Nelson Mandela African Institution of Science and Technology, P.O. Box 447, Arusha, Tanzania.

²Kenya Agricultural and Livestock Research Organization, P.O. Box 169-50100, Kakamega, Kenya.

³International Livestock Research Institute, P.O. Box 24384, Kampala, Uganda.

⁴Department of Livestock and Fisheries, Babati, Manyara Region, P.O. Box 310, Babati, Tanzania.

⁵The International Center for Tropical Agriculture, P.O. Box 6433, Morogoro, Tanzania.

⁶College of Agriculture and Veterinary Sciences, University of Nairobi, P.O. Box 29053-00625, Kangemi, Kenya.

⁷Masinde Muliro University of Science and Technology, P. O. Box 190-50100, Kakamega, Kenya.

⁸World Agroforestry Centre (ICRAF), P.O. Box 5689, Addis Ababa, Ethiopia.

Authors' contributions

This study was carried out in collaboration among all authors. Authors OPM, LAB and MAE designed the study, wrote the protocol and interpreted the data. Authors OPM, HA, DPN, LAB and MAE anchored the field study, gathered the initial data and performed preliminary data analysis. While Authors OPM and MAE managed the literature searches and produced the initial draft. Authors GC, EW and MFN reviewed and refined the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Aim: Crop residues from dual-purpose crops, particularly from coarse cereal and leguminous crops are by far the most important feed source available to smallholder dairy farmers in highlands and lowlands in Eastern Africa. Therefore, this study aimed to (1) determine the effect of urea and molasses pre-treatment on nitrogen gain, digestibility and rumen micro-biota of crop residues by *in vitro* culture; and (2) validate the effect of feeding pre-treated urea and molasses maize stover on feed intake and milk yield of dairy cows.

Methods: Fresh dry crop residue samples were collected in highlands and lowlands agro-ecological zones of Manyara region, Northern Tanzania and pre-treated with urea and molasses. The *in-vitro* culture experimental design was completely randomized block with 3 runs (replicates) and 3 crop residue treatments (control, urea, urea + molasses), with duplicates of 2 bottles per each treatment within a run. From the *in vitro analysis*, only maize stover had significant ($p \leq 0.05$) urea and molasses pre-treatment effect, and was therefore considered for comprehensive *in vitro* culture. The effect of urea and molasses pre-treatment of maize stover on intake and milk yield was validated in a feeding trial of Friesian cows in Siaya lowlands in Kenya.

Results: Pre-treatment of crop residues with urea and molasses resulted into significant ($p \leq 0.05$) improvements in chemical composition and fermentation products, but not gene copies of selected rumen microbes ($p \geq 0.05$), with exception of methanogens ($p \leq 0.05$). Urea and molasses pre-treated maize stover diet slightly improved milk yield and growth of dairy cows, reduced expenditure on labour with respect to feeding and the cost of producing milk and contributed to an increase in dry matter intake.

Conclusion: Despite the improvements in feeding value of maize stover, and other crop residues in general, with urea and molasses pre-treatment, the efficient utilization to desirable extent is still awaited.

Keywords: Crop residue; dairy cattle; feeding value; milk yield; molasses; Urea; validation.

1. INTRODUCTION

Smallholder dairying is important in sustaining livelihoods in Eastern Africa, where dairy cattle diets are forage based [1]. In the past, this would be provided through grazing, or more recently by growing planted forages mainly Napier grass [2]. However, as farm sizes decrease through inter-generational subdivision and farms intensify, farmers seek to maximize food security by growing food crops (cereals and legumes) alongside planted forage feeds [2]. For many small holder farmers, feeding dairy cattle over the dry season period when forages are scarce is a major challenge [3]. The inadequate forage feed availability and supplies is aggravated by seasonal variations in quantity and quality that causes fluctuations in animal nutrition and productivity throughout the year [4]. Hence, to bridge the feed gap, most small holder farmers mainly depend on crop residues to meet the nutrient requirements of the animals [5]. Crop residues are roughages, potentially rich sources of energy, as about 80% of their dry matter (DM) consists of polysaccharides, but are usually underutilized because of their highly lignified fibre, deficiency in mineral nutrients such as Nitrogen (N), Sulphur (S), Phosphorus (P) and Cobalt

(Co), which are essential to rumen microorganism function and their low digestibility [6,7]. Crop residues, such as maize stover, bean haulms, sunflower straw, pigeon pea haulms, rice straw, groundnut husks, sugarcane tops, wheat straw, etc., are abundant in the food crop growing areas in Eastern Africa, as largely underutilized by-product because of their low digestibility, which limits feed intake. These crop residue based diets in their natural form, cannot meet nutrient requirements of dairy cattle and often result in low milk production, sub-optimal reproductive performance and general poor health [8]. However, these poor quality roughages have the potential to improve nutritional value and animal feeding systems through employing different treatment strategies [5].

Urea-Molasses treatment is well documented and has, however, emerged as the method of choice for use at farm level in the tropics as it is best adapted to the conditions of smallholder farmers [9,10]. Moreover, fertilizer grade urea is readily available and relatively cheap compared to other chemical treatments with either aqueous or anhydrous ammonia. It is recognized that when animals are offered a low-nitrogen, high

fibre roughage diets, as with most cereal crop residues, one of the critical limiting nutrients is fermentable nitrogen (N) available to rumen microbes [11]. The use of urea-molasses is a convenient way to avoid excessive intake of urea N which would result in Ammonia-Nitrogen losses from the rumen, and will ensure an almost continuous supply of ammonia-Nitrogen, along with readily soluble carbohydrate for microbial growth [12]. The cost of feeding is a major component of the total cost of milk production, up to about 60-65% [13,14], and hence reduction of feeding cost needs to receive due emphasis.

The introduction of improved feeding practices, based on strategic supplementation of locally available forage-feed resources, especially during the dry season, is required not only to enhance milk production, but also to introduce a sustainable farming practice that will ensure a continuous supply of milk even during feed scarcity. Therefore, the use urea-molasses pre-treated crop residues for feeding and/or supplementing dairy cattle will have a positive effect, when inclusion in feed rations is justified both from the biological point of view and financial returns [15,16]. However, details of information on the incubation (pre-treatment) of crop residues with urea-molasses and utilization practices are not well documented for the study locations. Additionally, the inclusion levels, incubation period, cost effectiveness and utilization of crop residue pre-treated with urea-molasses for feeding lactating dairy cows has not been studied under the study region conditions. As a result, due consideration on assessment, development and evaluation of feeding options with urea-molasses pre-treated crop residue based feeding for milk production and other

animal performance indices is vital. The target end user is the smallholder dairy farmer in Eastern Africa, and it is hoped that this intervention will increase value of output without adding significant cost hence enhance adoption of urea-molasses pre-treatment for utilization of crop residues. Therefore, the objectives of this study were: (1) to pre-treat (incubate) crop residues with urea-molasses and assess the effect of the treatment on nitrogen gain, digestibility and rumen micro-biota by in-vitro culture; and (2) to assess the effect of feeding pre-treated molasses-urea maize stover on feed intake and milk yield of improved dairy cows in smallholder dairy farms.

2. MATERIALS AND METHODS

2.1 Urea and Molasses Pre-treatment of Dry Crop Residues and Analysis by *In-vitro* Culture

2.1.1 Fresh crop residue sample collection

Fresh dry crop residue samples were collected in highlands and lowlands agro-ecological zones in Mbulu and Karatu, which were the USAID's Feed The Future Africa RISING and SIMLESA II projects districts of Manyara region, Northern Tanzania. The crop residue samples consisted of maize (*Zea mays*) stover, bean (*Phaseola vulgaris*) haulms, sunflower (*Carthamus tinctorius*) husks, pigeon pea (*Cajanuscajan*) haulms, sorghum (*Sorghum bicolor*) stover and rice (*Oryza sativa*) straw (Fig. 1). Pre-treatment with urea and urea plus molasses and incubation was carried out at the Nelson Mandela African Institution of Science and technology (NM-AIST) laboratory, Arusha, Tanzania.



Plate 1a. Sunflower Husks



Plate 1b. Bean Haulms



Pate 1c. Rice Straw



Plate 1d. Pigeon pea Haulms



Plate 1e. Sorghum Stover



Plate 1f. Maize Stover

Fig. 1. Different types of dry crop residue samples collected from Mbulu highlands and Karatu lowlands

2.1.2 Substrate preparation and pre-treatment (incubation)

Fresh dry crop residue samples were sun-dried and chopped (pulverized) into ≤ 1 cm length and partitioned into three replicates of 0.5 kg. Samples were pre-treated by using two formulated solutions- urea solution (125 g of urea was dissolved by 0.5 L water) and urea plus molasses solution (125 g of urea and 10 mL of molasses were dissolved by 0.5 L water). The urea used for making solutions had 46% Nitrogen concentration. The crop residue samples were pre-treated with these two solutions: (a) urea pre-treatment- urea solution was mixed well with 0.5 kg crop residue samples; and (b) urea plus molasses pre-treatment- urea plus molasses solution was mixed well with 0.5 kg crop residue samples. Nylon bags (20 x 10cm with an average pore size of 50 μ m) were used for incubation. The urea and urea plus molasses pre-treated samples were properly labelled and incubated for 28, 45 and 90 days. Each sample upon completion of incubation period were oven dried in temperature of 70°C for 48 hours. The samples were then cooled for 3 hours and ground to pass through 2mm sieve. Fifty grams (50 gms) of samples were packed in a small clear zip plastic bags and shipped to the Institute of Sub-tropical Agriculture, Chinese Academy of Sciences in China for further analysis by *in-vitro* culture. Phytosanitary certificate and clearance for shipping of the samples was obtained from Kenya Plant Health Inspectorate Services and the Ministry of Agriculture in Nairobi, Kenya by the international Livestock Research Institute in Nairobi, who also met the shipping costs.

2.1.3 Experimental design for in-vitro culture

The in-vitro culture experimental design was completely randomized block with 3 runs

(replicates) and 3 crop residue treatments (control, urea, urea + molasses), with duplicates of 2 bottles for each treatment within a run.

2.1.4 *In vitro* incubation and sampling procedures

All animal procedures used in this study were reviewed and approved by the Animal Care Committee, Institute of Subtropical Agriculture, the Chinese Academy of Sciences, Changsha, China. Mixed rumen fluid from 3 healthy adult ruminally-cannulated Xiangdong black goats (25.0 se. = 2.0 kg average body weight) was used to prepare the inoculum for the *in vitro* culture fermentation. Goats were fed a total mixed ration containing 500g kg⁻¹ rice straw and 500g kg⁻¹ concentrate (554 g corn grain, 198 g wheat bran, 185 g soybean meal, 30 g soybean oil, 12 g calcium carbonate, 11 g sodium chloride, and 10 g premix with vitamins and microelements per kg of DM), offered twice per day at 08:00 and 18:00. Goats received 600 g/day of fodder and they had free access to water. Rumen contents were collected from the rumen before the morning feeding. Rumen inoculum was prepared by filtering the whole rumen contents through 4 layers of sterile cheese-cloth into a pre-warmed insulated bottle, then mixing it with artificial saliva [17], using a ratio of 1:4 (rumen fluid: saliva) to prepare the buffered rumen fluid. Substrate (1.2 g) was weighed into each 135 ml serum bottles in duplicate; and 60 ml buffered rumen fluid added under a stream of carbon dioxide (CO). The bottles were immediately sealed with butyl rubber stoppers, and incubations carried out at 39.5°C in an automated *in vitro* batch incubation system with venting pressure set at 10.0 kPa. The gas was automatically vented into a gas chromatograph (Agilent 7890 A, Agilent Inc., Palo Alto, California, USA) for measuring CH₄

and H₂ concentrations. The *in vitro* fermentation was terminated after 48 h or 72 h (for comprehensive *in vitro* culture) of incubation to collect liquid, solid and microbial samples. Chemical composition parameters determined consisted of gas production (GP), dry matter (% DM), dry matter digestibility (% DMD), crude protein (% CP), Metabolizable energy (ME, MJ Kg DM), crude fibre (CF) and Ash. ME was calculated from formula by [18], where $ME = 2.20 + 0.136 Gp + 0.057 CP$.

2.1.5 Comprehensive *in-vitro* culture of maize stover

In vitro-culture of all the crop residue samples in this study showed that only maize stover had significant ($P \leq 0.05$) urea and urea plus molasses pre-treatment effect, and was therefore considered for further comprehensive *in vitro* culture (fermentation). About 2 mL of liquid without particles was collected from each bottle and centrifuged at 15,000 g for 10 min at 4°C. The supernatant (1.5 mL) was acidified using 0.15 mL of 25% (w/v) meta-phosphoric acid, and stored at -20°C for analysis of volatile fatty acids (VFA) and ammonia. Microbial samples (1 mL × 3 replications) were collected after intense shaking of the bottle to ensure the samples included representative portions of liquid and particle fractions. Microbial samples were immediately frozen with liquid N₂ and stored at -80°C until DNA extraction. After sampling for VFA and DNA, the pH was measured immediately with a portable pH meter (Starter 300; Ohaus Instruments Co. Ltd., Shanghai, China). The residuals were filtered into pre-weighed Gooch filter crucibles, dried at 105°C to constant weight and weighed to determine degradation of incubated substrates and neutral detergent fiber (NDF).

2.2 Urea and Molasses Pre-treated Maize Stover on Intake and Milk Yield of Friesian Cows

2.2.1 Preparation urea and molasses pre-treated maize stover compacted block

The feeding trial was conducted in Siaya lowlands in Western region of Kenya between April and June 2019. Maize stover was pulverized (fine chopped), pre-treated using urea and molasses and incubated for 28 days before being compacted into 5 kg feed blocks. This was necessary for enhancing efficiency of handling and utilization, feeding value and controlled feeding during the trials. Three dietary treatment

were tested during the on-farm validation study and these included farmer-led feeding practice (FFP), urea plus molasses pre-treated maize stover block (MUMS), and urea pre-treated maize stover block (UMS). The diets were prepared *in situ* at the farms where the studies were conducted. Maize stover was pre-treated with urea and urea plus molasses and incubated on-farm for 28 days before compaction into feed blocks. Urea plus molasses pre-treated maize stover (MUMS) basal diet consisted of 10.0 kg DM pulverized/shredded (≤ 1.0 cm) maize stover. Then 200 grams of urea (N=46% grade for Kenya and Tanzania), 200 g ruminant salt, and 1.0 kg molasses dissolved in 5.0 L of water in a bucket. The liquid mixture was sprinkled on the shredded maize stover spread on a polythene sheet, then thoroughly mixed and incubated for 28 days in an airtight container before compacting for feeding. Urea pre-treated maize stover (UMS) basal diet was prepared with 400 g urea (N=46%) dissolved into 5 L of water and then sprinkled on pulverized/shredded (≤ 1.0 cm) maize stover (10 kg DM) spread on a polythene sheet. After ensuring a thorough well mixing of ingredients, the diet was transferred into a large airtight polythene bag. The mixture was incubated for 28 days so as to give ample time for urea to act on the straw. After 28 days the bags were opened and straw was ready for feeding but prior to feeding the urea pre-treated straw was aerated to remove any unreacted ammonia and compacted into feed block.

2.2.2 Dairy cows and their management

Twelve (12) farmers were purposively selected on the criteria of owning at least three milking cows, willingness to fully dedicate 2 milking cows to the experiment to the end, and acceptance of modest compensation for use of the animals. For each of the collaborating farms in the on-farm validation study, two lactating Friesian cows were selected based on similarity in their breed, milk yields, live body weights (mean 397.37 se. = 15.09 kg and were producing 9.45 se. = 0.46 kg of milk per day at the beginning of the trial) and 6-7 years in early-mid lactation period. The farmers were located in Siaya lowlands in Kenya and reared their dairy cows in intensive (stall feeding only) production system. The reason was that cows with the same yielding ability would likely show similar responses in milk yields.

2.2.3 Experimental design and feeding

Selected Friesian dairy cows were allocated in a three-period crossover design, following a

Table 1. The nutritive value of Napier grass, dry maize stover, pre-treated molasses-urea maize stover for validation feeding trials

Diet	GP 48h	DMD	DM	Ash	CP	Fat	Fibre	ME
Napier grass	229.68	61.85	89.13	17.32	9.42	2.8	29.4	8.12
Dry Maize stover	154.01	41.32	93.00	9.16	3.70	0.12	15.00	5.53
UMS	221.35	47.76	91.89	9.75	6.69	5.35	27.80	7.81
MUMS	228.67	47.67	92.38	9.18	10.77	8.27	29.33	8.10

GP = Total Gas Production at 48 hours; DMD = Dry Matter Digestibility (%); DM = Dry Matter (%); Ash = % Ash; CP = Crude Protein (%); Fat = Crude fat (%); Fibre = Crude Fibre (%); ME = Metabolizable Energy (MJ Kg DM); MUMS = Urea plus Molasses pre-treated maize stover; UMS = Urea pre-treated maize stover

sequence of dietary treatment administration of diet 1 (FFP), diet 2 (MUMS), and diet 3 (UMS). During the initial seven (7) days, the current farmer-led feeding practice (FFP) was administered by the farmer but monitored by the project data clerks at each of the twelve (12) collaborating farms, selected randomly from the cross sectional survey. This was because animal performance under farmer-led feeding practice was to be compared with improved pre-treated maize stover diets. For the FFP (diet 1), the animals were fed Napier grass (cut and carry) as basal feed with dairy concentrate/meal (2 kg/day) and mineral block, supplemented during milking time in the morning and evening prior to and during the experiment as positive control. The pre-treated urea and urea plus molasses maize stover basal diets 2 and 3 (UMS and MUMS) were offered to the cow free choice, with dairy concentrate/meal offered during milking time at rate of 2 kg/day/cow (morning and evening) and mineral block as supplementary feed. There was a 14 days' adaptation between two diets administration. The nutritive value of Napier grass, dry maize stover, pre-treated MUMS and UMS is given in Table 1. Milking was done twice a day in the morning (06 00 h) and in the afternoon (at 17 00 h). Milk yield was weighed and recorded every day throughout the study period. Data was collected daily by the corresponding author and trained government extension staff from the two areas on observational visit interviews and monitoring. Data on cow performance, feed offered, intake levels and refusals, and milk yield were recorded.

2.3 Data Analysis

The forage samples were analyzed in triplicate for dry matter (DM), neutral detergent fibre (NDF), acid detergent fibre (ADF), ether extract (EE) and nitrogen (N) content. The DM (Method 930.15), OM (Method 942.05), EE (Method 963.15) and N (Method 970.22) were analyzed according to published methodologies [19]. The NDF and ADF were assayed according to the

methods of [18], and expressed as inclusive of residual ash. Heat stable α -amylase was added during the NDF analysis. Neutral-detergent soluble (NDS) was calculated using the equation: NDS (g/kg DM) = (1000 - NDF, g/kg DM). Hemicellulose was calculated from NDF and ADF using the following equation: Hemicellulose (g/kg DM) = NDF-ADF (g/kg DM). The *in vitro* NDF degradation (NDFD) was calculated according to the following equation as described by [20,21]: NDFD (g/kg) = $(1 - (W_2 \times NDF_2) / (W_1 \times NDF_1)) \times 1000$. Where NDF₁ is NDF content in the substrate before incubation, NDF₂ is NDF content in the residue after 72 h incubation; W₁ is DM weight of substrate before incubation, W₂ is DM weight of residue after 72 h of incubation. The VFA concentration was measured according to the procedure described by [21], using a gas chromatograph (Agilent 7890 A, Agilent Inc., Palo Alto, California, USA). Ammonia concentration was measured according to [22]. DNA was extracted according to the protocol for pathogen detection of stool using a E.Z.N.A.TM Stool DNA Kit (Omega bio-tech, USA). The quantitative real time polymerase chain reaction (qPCR) was performed according to the procedure described by [23]. Forward primer (F) and reverse primer (R) were selected from the literatures for qPCR groups. A standard curve was generated using plasmid DNA containing the exact 16S/18SrRNA gene inserts and the standard curve met the following requirements ($R^2 > 0.99$, 90% $< E < 120\%$). The quantitative PCR assay was performed on a Light CyclerTM 480 (Roche Molecular Systems, Inc. USA) with a sample volume of 10 μ L that contained 5 μ L SYBER Green Mix (TaKara Inc., Dalian, China), 1 μ L of genomic DNA (10 ng/ μ L), 0.25 μ L of each primer and 3.5 μ L of ddH₂O. Comprehensive *in-vitro* analysis was performed on maize stover that had significant ($P \leq 0.05$) urea-molasses pre-treatment effect. The hexose fermented (HF), estimated net H₂ production relative to the amount of total VFA produced (R_{NH_2}), H₂ generated, H₂ utilized, H₂ recovery and fermentation efficiency (FE) was calculated by

the flow of reducing equivalences based on VFA and CH₄ produced using the equations described by [21] as follows: $R_{NH_2} = [2 \text{ (acetate + butyrate + isobutyrate)} - \text{(propionate + valerate + isovalerate)}]/\text{VFA}$. Total gas production (GP, ml gas/g DM) over the 72-h incubation was estimated from the cumulative pressure in the headspace of the bottle over time. Methane and hydrogen gas concentrations were measured each time the gas was vented from each bottle, thus CH₄ production at a particular incubation time was estimated from the values at the nearest two time points assuming a linear relationship. The fractional rates of total GP or CH₄ production were estimated using the Nonlinear Regressions Analysis Program (NLREG, version 5.4) [24], and calculated according to the equations described by [21]: $GP_t = Vf \times (1 - \exp(-kt)) \times (1 + \exp(b-kt))$. Where GP_t is the accumulated gas production at time t , Vf is the final asymptotic gas production (mmol/g), k is the fractional rate of gas production, b is the shape parameter. The data were the average of the two bottles per treatment within each run. The final data were analyzed using the general linear model procedure of SPSS 21.0 (Chicago, IL, USA) using a model that included the fixed effects of treatment ($n = 3$) and run ($n = 3$). Feeding validation data was also analyzed using multivariate analysis of variance (MANOVA) at 95% confidence level ($\alpha = 0.05$) as it allowed comparison of multiple dependent (response) variables and factors (independent) in the model. Descriptive statistics and tests of significance using least square difference were carried out. Statistical significance was considered at $p \leq 0.05$ with $0.05 \leq p \leq 0.10$ considered as a trend.

3. RESULTS

3.1 *In vitro* Analysis and Chemical Composition of Dry Crop Residues

In vitro culture analysis of the crop residue samples (Table 2), showed that nutritive value in terms of DMD was slightly higher for crop residues pre-treated with urea plus molasses (49.85% se. = 1.07) and lower for urea (45.56% se. = 1.08), as compared with molasses (47.90% se. = 1.20). Regarding chemical composition, crude protein content was higher for crop residues pre-treated with urea plus molasses (10.51% se. = 0.35) and lower for molasses (7.45% se. = 0.39) as compared with urea (9.05% se. = 0.35). Crude fibre was slightly higher for urea pre-treated crop residues

(32.44% se. = 0.97) as compared with urea plus molasses (29.51% se. = 0.96) and molasses (28.64% se. = 1.08). Further, *In vitro* analysis for chemical composition (Table 2) showed that total gas production after 48 hours of incubation was slightly higher in pigeon pea haulms (276.25 se. = 8.85 ml gas/g DM) and lower in sunflower hulls/husks (165.86 se. = 7.69 ml gas/g DM), as compared with the other crop residues. Dry matter digestibility was slightly higher in bean haulms (53.87% se. = 1.60) and lower in sunflower hulls/husks (33.98% se. = 1.26) as compared with the other crop residues. However, the dry matter content for all the crop residues evaluated was between 90.0 – 92.0%. The ash content was slightly higher in sunflower hulls/husks (13.04% se. = 1.29) and lower in pigeon pea haulms (6.22 se. = 1.48). Similarly, crude protein content was slightly higher in sunflower hulls/husks (12.85% se. = 0.41) and lowest in rice straw (5.45% se. = 0.60) in comparison with the other crop residues. Further, crude fat content was also higher in sunflower hulls/husks (8.91% se. = 0.98) and lower in sorghum stover (3.66% se. = 1.27) and pigeon pea haulms (3.79% se. = 1.13). Crude fibre was slightly higher in bean haulms (36.41% se. = 1.44) and lowest in sorghum stover (25.24% se. = 1.46). Metabolizable energy (MJ/Kg DM) was slightly higher in pigeon pea haulms (9.55% se. = 0.29) and lower in sunflower hull/husks (6.04% se. = 0.25). Sunflower hulls/husks, with lower total gas production, dry matter digestibility and metabolizable energy, had higher ash, crude protein and crude fat contents. Contrastingly, pigeon pea haulms, with higher total gas production and metabolizable energy, had lower ash and crude fat content.

3.2 Comprehensive *In vitro* Analysis of Urea and Urea Plus Molasses Pre-treated Maize Stover

3.2.1 Chemical composition

Comprehensive *in-vitro* analysis for chemical composition of maize stover pre-treated using urea and urea plus molasses (Table 4) revealed that the treatment effect was significant ($p \leq 0.05$) on Neutral detergent fibre (NDF, g/kg DM), Neutral detergent soluble (NDS, g/kg DM), Acid detergent fibre (ADF, g/kg DM), Hemicellulose (g/kg DM), Acid detergent lignin (ADL, g/kg DM) and Total nitrogen (TN, g/kg DM), but not Ether extracts (EE, g/kg DM, $P \geq 0.05$). Pre-treatment decreased NDF more with urea plus molasses (647 g/kg DM), and less with urea (827 g/kg DM), add as compared with the control (831 g/kg DM).

Similarly, pre-treatment decreased ADF more with urea plus molasses (543 g/kg DM), and less with urea (605 g/kg DM), as compared with control (744 g/kg DM). However, pre-treatment increased NDS more with urea plus molasses (357 g/kg DM), and less with urea 9176 g/kg DM), as compared with control (169 g/kg DM). Hemicellulose increased more with urea (222 g/kg DM), and less with urea plus molasses (100 g/kg DM), as compared with control (87.4 g/kg DM). ADL decreased more with urea (64.7 g/kg DM), and less with urea plus molasses (112 g/kg DM) as compared with control (131 g/kg DM). Total N (TN) was increased more with urea plus molasses (15.7 g/kg DM), and less with urea (11.8 g/kg DM) as compared with the control (7.6 g/kg DM).

3.2.2 Ruminal fermentation characteristics

Comprehensive *in-vitro* analysis for ruminal fermentation characteristics of maize stover pre-treated using urea and urea plus molasses (Table 5) showed that the treatment effect was significant ($p \leq 0.01$) on dry matter digestibility (% DMD), Ammonia (NH_4^+) concentration (mM – micro Mols), pH, Total volatile fatty acids (VFA, mM) and Molar proportion of specific VFAs (Acetate, Butyrate, Isobutyrate, Isovalerate, Propionate) with the exception of Valerate ($P = 0.088$), Acetate to propionate ratio and estimated net Hydrogen production relative to the amount of total VFA produced ($R_N\text{H}_2$, mol/100mol VFA). DMD increased more with pre-treatment with urea plus molasses (61.2%), and less with urea (42.6%), as compared with the control (35.4%). Ammonia (NH_4^+) concentration was increased with pre-treatment with urea plus molasses

(10.3mM), but slightly decreased with urea (7.10 mM), as compared to with the control (7.28mM). pH was not affected by pre-treatment of maize stover with either urea or urea plus molasses. However, total VFAs concentrations were increased more with pre-treatment using urea with plus molasses (72.1mM), and less with urea (63.4mM), as compared with the control (55.6mM). Molar proportions of individual VFAs varied with pre-treatment using with either urea or urea plus molasses, with the exception of Valerate. Molar proportion of Acetate was slightly decreased with and urea (71.2mol) and urea plus molasses (67.9mol) pre-treatment than with the control (72.2mol). However, molar proportion of Propionate add was slightly increased with pre-treatment using urea (24.1mol) and urea plus molasses (25.4mol), add as compared with the control (22.7mol). Molar proportions of Butyrate and Isobutyrate were slightly decreased with pre-treatment with using urea (3.57mol and 0.37mol, respectively), but increased with urea plus molasses (5.17mol and 0.51mol, respectively), as compared with the control (3.98mol and 0.39mol, respectively). Molar proportion of Isovalerate add was slightly increased with pre-treatment using with urea (0.40mol), but more with urea plus molasses (0.64mol), as compared with the control (0.38mol). The ratio of Acetate to Propionate was decreased less with pre-treatment using with urea (2.96), but more using with urea plus molasses (2.68), as compared with the control (3.19). Similarly, the estimated net hydrogen produced relative to the amount of total VFAs produced ($R_N\text{H}_2$) was decreased with urea (126mol) and urea plus molasses (121mol) pre-treatments as compared with the control (130mol).

Table 2. The effect of pre-treatment with molasses, urea and urea plus molasses on chemical composition of crop residues from high and low altitude areas in Tanzania

Chemical Composition	Molasses		Urea		Urea + Molasses	
	Mean	se	Mean	se	Mean	se
Gas_48 hrs	238.83	7.32	221.57	6.59	234.79	6.54
DMD (%)	47.90 ^a	1.20	45.56 ^a	1.08	49.85 ^b	1.07
DM (%)	91.89	0.19	91.44	0.17	91.74	0.17
Ash (%)	10.54	1.23	9.96	1.11	11.72	1.10
CP (%)	7.45 ^a	0.39	9.05 ^b	0.35	10.51 ^c	0.35
Fat (%)	5.06	0.93	6.18	0.84	6.00	0.83
Fibre (%)	28.64 ^a	1.08	32.44 ^b	0.97	29.51 ^{ab}	0.96
ME	8.35	0.24	7.81	0.21	8.24	0.21

Gas = Total gas production after 48hr (ml gas/g DM); DMD = Dry matter digestibility (%); DM = dry matter (%); CP = Crude protein (%); Fat = Crude fat (%); Fibre = Crude fibre (%); ME = Metabolizable energy (MJ/Kg DM); SEM-Standard Error of Mean; ^{abcde}-Means with different superscript letters were significantly different ($P \leq 0.05$)

Table 3. The effect of pre-treatment with urea and molasses on chemical composition of dry crop residues from high and low altitude areas in Tanzania

Chemical Composition	Bean haulms		Maize stover		Pigeon pea haulms		Rice straw		Sorghum stover		Sunflower hulls	
	Mean	se	Mean	se	Mean	se	Mean	se	Mean	se	Mean	se
Gas_48 hrs	264.02 ^{cd}	9.79	221.81 ^b	10.36	276.25 ^d	8.85	219.59 ^b	11.17	242.85 ^{bc}	9.94	165.86 ^a	7.69
DMD (%)	53.87 ^d	1.60	46.94 ^b	1.69	51.61 ^{bc}	1.45	47.22 ^b	1.82	53.01 ^{bc}	1.62	33.98 ^a	1.26
DM (%)	90.70	0.23	91.84	0.28	92.04	0.21	92.18	0.28	91.67	0.26	91.72	0.18
Ash (%)	9.92 ^{ab}	1.64	9.45 ^{ab}	1.74	6.22 ^a	1.48	16.59 ^c	1.87	9.19 ^{ab}	1.67	13.04 ^{bc}	1.29
CP (%)	9.39 ^c	0.53	7.87 ^b	0.56	7.23 ^b	0.48	5.49 ^a	0.60	11.17 ^d	0.53	12.85 ^e	0.41
Fat (%)	5.37 ^a	1.25	5.11 ^a	1.32	3.79 ^a	1.13	7.63 ^{ab}	1.43	3.66 ^a	1.27	8.91 ^b	0.98
Fibre (%)	36.41 ^a	1.44	29.88 ^b	1.52	30.10 ^b	1.30	28.22 ^{ab}	1.64	25.24 ^a	1.46	31.32 ^b	1.13
ME	9.18 ^{cd}	0.32	7.80 ^b	0.34	9.55 ^d	0.29	7.76 ^b	0.36	8.47 ^{bc}	0.32	6.04 ^a	0.25

Gas = Total gas production after 48hr (ml gas/g DM); DMD = Dry matter digestibility (%); DM = dry matter (%); CP = Crude protein (%); Fat = Crude fat (%); Fibre = Crude fibre (%); ME = Metabolizable energy (MJ/Kg DM); SEM-Standard Error of Mean; ^{abcde}-Means with different superscript letters were significantly different ($P \leq 0.05$)

Table 4. The effect of urea and urea plus molasses pre-treatments on the chemical composition of maize stover

Chemical composition	Treatments			SEM ¹	P value
	Control	Urea	Urea+molasses		
NDF, g/kg DMI	831 ^a	827 ^a	643 ^b	8.6	<0.001
NDS,	169 ^b	176 ^b	357 ^a	8.6	<0.001
ADF,	744 ^a	605 ^{ab}	543 ^b	13.4	0.024
Hemicellulose	87.4 ^b	222 ^a	100 ^b	7.5	0.015
ADL,	131 ^a	64.7 ^b	112 ^{ab}	9.3	0.041
TN,	7.6 ^c	11.8 ^b	15.7 ^a	0.36	<0.001
EE,	85.8	106	95.7	15.8	0.073

¹NDF, neutral detergent fiber; NDS, neutral detergent soluble; ADF, acid detergent fiber; ADL, acid detergent lignin; TN, total nitrogen; EE, ether extract. Units = g/kg DM, ¹Standard error of means

Table 5. The effect of urea and urea plus molasses pre-treatments on the *in vitro* ruminal fermentation characteristics for maize stover

Ruminal fermentation characteristics	Treatments			SEM ¹	P value
	Control	Urea	Urea+molasses		
DMD,%	35.4 ^c	42.6 ^b	61.2 ^a	0.89	<0.001
NH ₄ ⁺ ,mM	7.28 ^b	7.10 ^b	10.3 ^a	0.36	0.006
pH	6.61	6.57	6.63	0.07	0.795
Total VFA, mM	55.6 ^c	63.4 ^b	72.1 ^a	4.31	0.005
Molar proportion of individual VFAs, mol/100mol					
Acetate	72.2 ^a	71.2 ^a	67.9 ^b	0.52	0.009
Butyrate	3.98 ^b	3.57 ^b	5.17 ^a	0.28	0.036
Isobutyrate	0.39 ^b	0.37 ^b	0.51 ^a	0.03	0.054
Isovalerate	0.38 ^b	0.40 ^b	0.64 ^a	0.05	0.033
Propionate	22.7 ^b	24.1 ^a	25.4 ^a	0.33	0.011
Valerate	0.36	0.30	0.41	0.03	0.088
Acetate to propionate ratio	3.19 ^a	2.96 ^b	2.68 ^c	0.06	0.008
R _N H ₂ , mol/100mol VFA	130 ^a	126 ^b	121 ^c	1.00	0.007

¹DMD, dry matter degradation; VFA, volatile fatty acids; R_NH₂, estimated net H₂ production relative to the amount of total VFA produced. ¹Standard error of means

3.2.3 Gas production

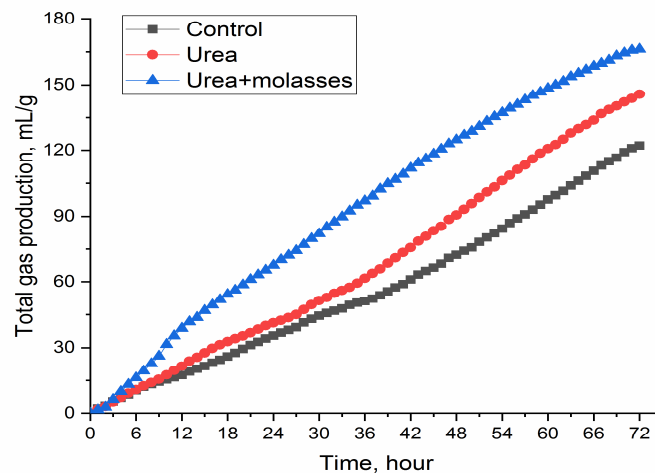
Comprehensive *in-vitro* analysis for total gas production from maize stover pre-treated using urea and urea plus molasses (Table 6) showed that the treatment effect was significant ($p \leq 0.05$) on total gas produced per dry matter (mL/g DM), total gas produced per digestible dry matter (mL/g DDM) and Methane gas produced per digestible dry matter after 72 hours of *in vitro* incubation (72h CH₄ mL/g DDM). However, the treatment effect had no significant influence ($p \geq 0.05$) on the fractional rate of total gas production per hour (k_{GP}/h), the fractional rate of methane gas production (K_{CH_4}/h), Hydrogen gas produced per digestible dry matter after 72 hours of *in vitro* incubation (72h H₂ mL/g DDM) and the fractional rate of hydrogen gas production per hour (k_{H_2}/h). Total gas production was increased more with

pre-treatment using urea plus molasses (166mL/g DDM) and less with urea (144mL/g DDM) as compared with the control (122mL/g DDM). However, total gas produced per digestible dry matter was decreased more with pre-treatment using urea plus molasses (272mL/g DDM) and less with urea (341mL/g DDM) as compared to the control (344mL/g DDM). Methane gas production per digestible dry matter was decreased with pre-treatment (31.7mL/g DDM), but not with urea (42.9mL/g DDM) as compared with the control (42.0mL/g DDM). There was a steady increase (Fig. 2) in total gas production up to 72 hours of *in-vitro* incubation for the three treatments (control, urea and urea plus molasses). However this increase in total gas production was higher with urea plus molasses, followed by urea, as compared to with the control (Fig. 2).

Table 6. The effect of urea and urea plus molasses pre-treatments on the *in vitro* gas productions for maize stover

<i>In vitro</i> gas production	Treatments			SEM ¹	P value
	Control	Urea	Urea+molasses		
Total gas, mL/g DM	122 ^c	144 ^b	166 ^a	4.40	<0.001
Total gas, mL/g DDM	344 ^a	341 ^a	272 ^b	14.70	0.041
k _{GP} , /h	0.013	0.015	0.017	0.006	0.800
72h CH ₄ , mL/g DDM	42.0 ^a	42.9 ^a	31.7 ^b	2.25	0.044
k _{CH₄} /h	0.046	0.050	0.056	0.014	0.790
72h H ₂ , mL/g DDM	0.08	0.09	0.06	0.013	0.273
k _{H₂} /h	0.101	0.109	0.118	0.043	0.930

¹k_{GP}, the fractional rate of total gas production; k_{CH₄}, the fractional rate of CH₄ production; k_{H₂}, the fractional rate of H₂ production. ¹Standard error of means, DDM, digestible dry matter

**Fig. 2. Variation in the total gas production during the 72 h *in vitro* ruminal incubation of maize stover pre-treated with urea and urea plus molasses****Table 7. The effect of urea and urea plus molasses pre-treatments on the gene copies of selected microbial groups (Log₁₀ copies/g DM *in vitro* rumen contents)**

Gene copies of selected Microbes ¹	Treatments			SEM ²	P value
	Control	Urea	Urea+molasses		
Fungi	7.27	7.11	7.08	0.534	0.471
Bacteria	9.73	9.80	9.79	0.114	0.566
Protozoa	7.46	7.69	7.82	0.244	0.201
Methanogen	8.70 ^{ab}	8.62 ^b	8.77 ^a	0.036	0.015
<i>Methanobacteriales</i>	7.44	7.36	7.61	0.233	0.336
<i>Metanobrevibacteria</i>	7.39	7.37	7.61	0.254	0.389
<i>Methanomicrobiales</i>	5.63	5.73	5.74	0.205	0.647
<i>Prevotella</i> spp.	9.26	9.27	9.30	0.119	0.788
<i>R. albus</i>	6.13	6.11	6.06	0.172	0.718
<i>R. flavefaciens</i>	6.18	6.24	6.19	0.526	0.920
<i>F. succinogenes</i>	8.21	8.05	8.39	0.366	0.663
<i>S. ruminantium</i>	8.82	9.15	9.05	0.376	0.419

¹16SrRNA gene copies were measured for bacteria and methanogen, 18SrRNA gene copies were measured for protozoa and *Prevotella* spp., and the multiple alignments of 18SrRNA and ITS1 gene copies were measured for fungi. ²Pooled standard error of means

Table 8. Effects of feeding urea and urea plus molasses pre-treated maize stover feed blocks on dry matter intake and milk yield of Friesian dairy cows at farm level

Source of feeds	DMI kg/cow/day	Cost/kg DM USD	Milk yield L/cow/day	Cost/Litre USD
FFP	10.26 ^a	0.0165	12.70 ^a	0.018
UMS	11.18 ^b	0.0140	13.34 ^a	0.016
MUMS	11.42 ^c	0.0150	13.51 ^a	0.017

Means with different superscript letters were significantly different ($p < 0.05$)

Table 9. Effects of feeding UMS and MUMS feed blocks on changes in dry matter intake, live body weight gain and milk yield of Friesian dairy cows at farm level

Feed source	Milk yield L/cow/day	% milk increase from initial milk production	DMI increase kg/cow/day (%)	LBW gain (%)
UMS	13.34	38.5	5.7	5.1
MUMS	13.51	40.5	4.6	6.5
LSD	0.74	12.5	0.6	0.1
Significance	Ns	***	***	***

Means with different superscript letters were significantly different ($p < 0.05$)

3.2.4 Gene copies of selected microbes

Comprehensive *in-vitro* analysis to determine gene copies of selected microbes from maize stover pre-treated using urea and urea plus molasses (Table 7) showed that the treatment effect was not significant ($p \geq 0.05$) on the 16S/18SrRNA/ITS1 gene copies of total Bacteria, Fungi, Protozoa, *Prevotella spp.*, *Ruminococcus albus*, *Ruminococcus flavefaciens*, *Fibrobacter succinogenes* and *Selenomonas ruminantium*. However, pre-treatment with urea and urea plus molasses had a significant effect ($p \leq 0.05$) on the 16SrRNA gene copies of total Methanogens, but not ($p \geq 0.05$) the 16SrRNA gene copies of specific Methanogens (Methanobacteriales, Methanobrevibacteria and Methanomicrobiales). Pre-treatment with urea plus molasses tended to increase total the 16SrRNA gene copies of total Methanogens (8.77 copies/g DM), but pre-treatment with urea tended to decrease the 16SrRNA gene copies of total Methanogens (8.62 copies/g DM), as compared with the control (8.70 copies/g DM).

3.3 Feeding Value of Urea and Molasses Pre-treated Maize Stover in Friesian Cows

Tables 8 and 9 present the effects of feeding Napier grass (FFP), urea (UMS) and urea plus molasses (MUMS) based feed block on dry matter intake (DMI) and milk yield of Friesian dairy cows. Milk yield for cows fed compacted UMS and MUMS feed block, dairy meal and mineral block supplement (test diets) and

chopped Napier grass, dairy meal and mineral block supplement (FFP as positive control) was not found significantly different ($p \geq 0.05$). However, UMS and MUMS based feed block showed better milk yield as compared to FFP. Change in milk yield (percent milk increase) from the initial milk production was higher (38 - 41%) for cows fed the UMS and MUMS feed block. Percent (%) increase in dry matter intake from the initial DMI was higher (4.50 – 6.00%) with cows fed the UMS and MUMS feed block. Percent (%) increase in body weight gain from the initial body weight was higher (5.00 – 7.00%) with cows fed the UMS and MUMS feed block. Table 9 presents also the cost of dry matter including that of producing a litre of milk from UMS and MUMS feed blocks and FFP. The cost of dry matter was higher with FFP by about USD 0.0165 as compared to that of UMS (0.0140) and MUMS (0.0150) feed blocks. The cost of producing a litre of milk from MUMS feed block was cheaper by about USD 0.002 per litre as compared producing from FFP. While the cost of producing a litre of milk from MUMS feed block was cheaper by about USD 0.001 per litre of milk.

3.4 Farmer Perceptions on Feeding Value of UMS and MUMS Feed Block in Feeding Dairy Cows

All farmers participating in the focused group discussion (FGD) agreed that UMS and MUMS feed block was necessary for improved dairy production. The farmers were convinced that the feed blocks addressed issues of fodder shortage

and nutrients levels. The blocks were observed as fast in making the cows full. They also created a thirst and the cows drunk almost 3 times more than usual amounts of water. This led to increased milk yield per cow per day. Dairy cattle feeding on feed blocks reduced the quantity of daily Napier grass requirement, time and labour involved in acquiring Napier grass. Farmers who used the UMS and MUMS feed block stated they had more free time for other farm activities and to attend to community meetings. The feed blocks were easy to handle, hence even the elderly, women and youth were convinced they could venture into dairy farming. Feeding dairy cattle with the feed blocks reduced the unnecessary stress caused by high labor turnover among livestock workers. On level of technology (compacted urea and urea plus molasses feed block) satisfaction, 36% farmers were highly satisfied while 64% were satisfied with feeding value. Dairy cows fed UMS and MUMS feed blocks were increased in body weight, looked healthy, ate to their fill, drunk enough water, laid down to rest, had an upward milk yield trend and an observed increase in milk cream in all the units where on farm trials were carried out. Farmers explained that cow milk increased from as low as 9 litres per cow per day to an average of 13 litres daily when cows were fed the feed blocks. When the blocks were finished and cows re-introduced to Napier grass, the average milk yield dropped back to the initial amounts.

4. DISCUSSION

Crop residues from dual-purpose crops, particularly from coarse cereal and leguminous crops, are by far the most important feed source available to smallholder dairy farmers in highlands and lowlands in Kenya and Tanzania. These crop residues were fibrous parts of crops that remain after those edible to human beings have been removed, as similarly reported by [7]. On average about 50–60% of total local feed resource in smallholder farms in Kenya and Tanzania obtained from crop residues, similar to findings by [7] in India. This study found considerable variation in the quality of urea and molasses pre-treated stover and straws in terms of chemical composition, dry matter digestibility as well as metabolizable energy, among different rice, sorghum, sunflower, maize, beans and pigeon pea crop residues across high and low altitude areas of Kenya and Tanzania. The choice of supplementing stovers/straws with forages and concentrates has previously been

reported to have a positive impact on animal productivity [1], while chemical treatment with urea and urea plus molasses had proven positive effect on digestibility and milk yield in dairy cows [6]. Fibre is essential in ruminants for rumination, saliva flow, rumen buffering, health of the rumen wall and high butterfat in milk [25]. The fibrous cell wall consists of hemicelluloses, cellulose and lignin, some of which may be digested by the rumen microbes [12]. High cell wall content increases rumination time and is associated with a decreased efficiency of conversion of metabolizable energy to net energy [12]. The rumen has a wide-range of fibrolytic microbial groups that degrade fibre. Fungi, protozoa and fibrolytic bacteria (i.e. *Ruminococcus albus*, *Ruminococcus flavefaciens* and *Fibrobacter succinogenes*) are active fiber degraders [26]. These rumen microbes were enhanced in this study through pre-treatment of the crop residues with urea and urea plus molasses.

Comprehensive in vitro culture was performed on maize stover as animal feed due to the fact that maize is a staple food crop grown in the highlands and lowlands areas where the study was performed, and also showed significant treatment effect as compared with the other crop residues. Rumen fluid pH was not altered among the treatments, and the values were stable at pH 6.50 to 6.70. This ruminal pH ranges from 6.5 to 6.7 was appropriate, according to [5,27], the optimum level of pH in the rumen for microbial digestion of fiber and protein should be 6.5 to 7.0 when fed mostly on roughages. The NH_4^+ values for rumen fermentation tended to be higher with urea plus molasses pre-treatment as compared with urea alone and controls. Ruminal NH_4^+ is an important nutrient in supporting efficient rumen fermentation [5,27]. Ruminal NH_4^+ concentrations in this study ranged from 7.10 to 16.4mM, which contrasted with the report of [27], who proposed a range from 13.6 to 17.6mM. The total VFA, acetic acid, propionic acid, butyric acid proportions, and acetic acid to propionic acid ratio showed that there were significant differences in VFA concentrations or molar proportions of VFA ($p < 0.05$), contrasting findings were obtained by [27]. The total VFA concentrations in all of the treatments ranged from 0.30 to 72.50mM and were lower than those reported by [5,27,28]. However, the proportions of acetate, propionate and butyrate in this study were in agreement to findings by [12]. These proportions of volatile fatty acids (acetate, propionate and butyrate) were not affected very much by urea pre-treatment as compared to urea

plus molasses, similar to findings reported by [20,29].

Results demonstrated that the feeding of urea and molasses pre-treated maize stover feed block improved dairy cattle performance in terms of Milk yield and growth rate. These results were supported through similar works by [13,14,27]. The explanation for these improved performance was because of utilization of improved crop residue with better feeding value as similarly reported by [6] that urea in the maize stover based feed blocks boosted the non-protein nitrogen level of the crop residues. This, perhaps could have provided alkali effect when compacted, which helped to break down the ligno-cellulose bond of the crop residues. Secondly, [12] explained that the feeding of pre-treated urea plus molasses feed block was generally allowed for synchronized supply of nutrients to microbes resulting in the synergy between nutrients demand of rumen microbes and the release of adequate levels of the nutrients bringing stability in the rumen ecosystem for optimal fermentation. In the feeding validation experiment, milk yield from animals fed maize stover alone was lower than the feed blocks and Napier grass. These findings were similar to those of obtained by [10], and suggests that maize stover alone could be the dry season diets to cope with feeds scarcity. Further, pre-treated crop residue feed blocks could be used for dry season supplementation in smallholder dairy cattle intensive feeding systems. The feed blocks as expressed by farmers were balanced and one expected improved supply of nutrients. Secondly, the feed blocks reduced feed wastage thus were efficient in delivery of nutrients. Thirdly, by feeding feed blocks the expenditure on labour with respect to feeding was reduced. These perceptions were supported by [30] who demonstrated that feeding feed blocks reduced labour with respect to feeding by 30-40%, where it took 20-30 minutes to feed 20 animals as opposed to hours of feeding the same animals drudgery being experienced in cutting, collecting and transportation of huge loads from roadsides. Milk yield from animals fed UMS and MUMS was almost similar to FFP. This study confirmed the findings by [10], who suggested that these urea and urea plus molasses pre-treated supplementary feeding diets should be dry season diets to cope with feeds scarcity rather than for high yield. Results from this study on higher values for MUMS as compared with UMS were in agreement with [10,14].

5. CONCLUSION

Improving nutritive value of feed resources, especially crop residues using urea and molasses is relatively important to smallholder dairy farmers in utilizing the crop residue feed resource effectively. Pre-treatment of crop residues such as maize stover with molasses and urea resulted into significant improvements chemical composition (total gas production, CP, ME, Ash, EE, Fat, Fibre) and fermentation products (volatile fatty acids, propionate, butyrate, and ammonia). Urea and molasses pre-treated maize stover based feed blocks improved milk yield and growth performance of dairy cattle, reduced expenditure on labour with respect to feeding, the cost of producing milk and contributed to increase in dry matter intake. However, despite the improvements in feeding value of crop residues through pre-treatment with urea and molasses, the efficient utilization to desirable extent is still awaited.

ETHICAL APPROVAL

All procedures performed in study involving human and animal participants were in accordance with the ethical standards of the researcher institutional (KALRO and NM-AIST) and/or national agricultural research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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