



Research Article

IN VITRO EVALUATION OF SUGARCANE BAGASSE TREATED WITH DIFFERENT LEVEL OF UREA AND MOISTURE

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Received: March 24, 2017; Revised: April 11, 2017; Accepted: April 12, 2017; Published: April 24, 2017

Abstract-The present study was conducted to evaluate the graded level of urea and moisture treatments to enhance the nutritive value of SCB. SCB was treated with 3.5, 4.0 and 4.5 % of urea at 40, 50 and 60 % levels of moisture in a 3×3 factorial arrangement and ensiled for a period of 21 days. Samples were analysed further proximate analysis and *In vitro* gas production parameters. The chemical composition of SCB treated with 3.5 % urea at 40% moisture level enhanced CP, digestibility of DM and OM and reduction in fiber fractions. IVDMD and IVOMD, ME, total volatile fatty acids, total nitrogen and ammonia levels were significantly ($P<0.05$) increased as compared to untreated SCB. On holistic view, treatment of SCB with 3.5% urea and 40% moisture seems to be optimum and best for ruminant production system.

Keywords- Chemical composition, *in vitro* digestibility, Moisture, Sugarcane bagasse, Urea

Citation: Patel V.R., et al., (2017) *In vitro* Evaluation of Sugarcane Bagasse Treated with Different Level of Urea and Moisture. International Journal of Agriculture Sciences, ISSN: 0975-3710 & E-ISSN: 0975-9107, Volume 9, Issue 19, pp.-4198-4200.

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Academic Editor / Reviewer: Dr J. B. Kathiriya, Ajaykumar Sureshbhai Patel, Dr Kirit R Bhedi, Dr Amrita A Vasava

Introduction

Adequate supply of feed and fodder to livestock is one of the major challenges faced by the India in current scenario. To bridge the gap between demand and supply of feed, use of unconventional and agro-industrial byproducts has been tried with special concern to alleviate their limitations, which are poor in nitrogen content, highly lignified resulting lower digestibility. Sugarcane bagasse is a secondary byproduct of sugar cane factory belongs to these characteristic. To improve the nutritive value of SCB, it is important to breakdown the linkages among cellulose and lignin by mechanical, chemical and biological or combination of these methods with increased nitrogen content. Urea treatment is an applicable technique for improving the quality of crop residues by increasing nitrogen content and nutrient digestibility and thereby animal performance [1]. Therefore, the present study was carried out to assess the changes in chemical composition, *in vitro* substrate degradability and fermentation characteristics of SCB treated with different levels of urea and moisture.

Material and Methods

Preparation of substrate for treatments and analysis

Samples of SCB were collected from different sugarcane factories of South Gujarat and pooled to get a representative sample. Representative samples were dried in oven at 90° C and grounded to pass in 1 mm sieve. SCB was treated with 3.5, 4.0 and 4.5 % urea at 40, 50 and 60% moisture level (3 x3) and ensiled for 21 days in polyethylene bags. At the end of ensiling period the samples were taken out from the bags, sundried for 2 days to eliminate free ammonia and prepared for further analysis [2] and fibre fractions [3].

Feeding regimen and collection of rumen liquor

Rumen liquor was collected from male Surti buffalo fed with basal diet including SCB at Livestock Research Station, NAU, Navsari. Rumen liquor was collected by

stomach tube in early morning before feeding and watering in to pre warmed thermo-flask, immediately filtered with muslin cloth under CO₂ bubbling at 39° C to get strained rumen liquor (SRL), which was used as inoculum and incubating medium.

Methodology

The incubations were carried out in 100 ml calibrated glass syringes [4]. 200 mg of dried substrate was placed into the bottom of the glass syringe without sticking to the sides of the syringe. The piston was lubricated with petroleum jelly and pushed inside the glass syringe. 30 ml of buffered rumen fluid was dispensed in each syringe and clamped with gentle shaking to mix the content. Syringes were placed vertically (upright) in stand and record the initial reading. The syringes were kept in an incubator at 39°C for 24 hrs of period with intermittent shaking. Record the reading by the displacement of the piston with gas produced during the incubation period and corrected with blank (SRL without substrates).

Nutrient utilization

The DM digestibility was estimated by transferring of syringe contents to a spoutless beaker by repeated washing with 100 ml (NDS) neutral detergent solution [3]. The flask content was refluxed for 1h and filtered through pre-weighed Gooch crucibles to arrive at DM residue. The *in vitro* degradable organic matter in the rumen (IVOMD) was calculated as the amount of substrate OM incubated minus the amount of substrate recovered as residue after NDS treatment: TDOMR = [(Initial OM of feed taken for incubation - NDF residue x100) / (Initial OM of feed taken for incubation)]. The partitioning factor (PF) was calculated as the ratio of IVOMD (mg) to gas volume (ml) produced from it during 24h of incubation: PF = IVOMD mg/ml of total gas produced [5]. Metabolizable energy was calculated as ME (MJ/kg DM) = 2.20 + 0.136× gas produced (ml/200 mg DM) + 0.0057×CP (g/kg DM) + 0.0029× EE²(g/kg DM) [4].

Statistical analysis

The data generated were analyzed for their statistical significance using Statistical Package for the Social Sciences (SPSS, version 20.0 Chicago, USA). Data were analyzed using one-way ANOVA to distinguish the impact of different dietary treatments. The effects were considered to be significant at $P < 0.05$ and declared as trend/tendency at $0.05 < P < 0.10$.

Results and Discussion

Proximate compositions and fibre fractions

Chemical composition of treated SCB is presented in [Table-1]. The variation among the treatment group was comparable ($P > 0.05$) although the differences in CP content was because of graded level of urea, which is good source of nitrogen along with role to breakdown the lignocelluloses bonds. Treated SCB showed a reduction ($P > 0.05$) in the values of fiber fractions i.e. NDF, ADF and HC. It might be due to partial solubilization of hemicelluloses by urea-moisture treatment. The results obtained for chemical composition of urea treated SCB were in agreement with earlier reports [6, 7].

Table-1 Effect of urea and moisture treatment of sugarcane bagasse proximate and fibre fractions

| Moisture % | 0 | 40 | | | 50 | | | 60 | | |
|------------|---------------------|---------------------|----------------------|----------------------|----------------------|---------------------|--------------------|---------------------|----------------------|--------------------|
| Urea % | 0 | 3.5 | 4.0 | 4.5 | 3.5 | 4.0 | 4.5 | 3.5 | 4.0 | 4.5 |
| Groups | Control | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9 |
| DM% | 97.53 ^{ba} | 97.74 ^{ba} | 97.79 ^{ba} | 97.56 ^{ba} | 97.35 ^b | 97.79 ^{ba} | 97.91 ^a | 97.79 ^{ba} | 97.62 ^{ba} | 97.89 ^a |
| OM% | 90.57 | 52.51 | 51.88 | 49.60 | 48.60 | 38.55 | 48.71 | 39.17 | 43.94 | 40.70 |
| CP% | 3.62 ^{ba} | 10.06 ^c | 11.36 ^{bac} | 11.44 ^{bac} | 10.85 ^{bac} | 11.87 ^{ba} | 12.70 ^a | 9.79 ^c | 11.38 ^{bac} | 12.20 ^a |
| EE% | 1.10 | 1.05 | 1.02 | 1.06 | 0.85 | 1.12 | 0.81 | 0.89 | 1.19 | 1.13 |
| Total Ash% | 2.47 ^{ba} | 2.26 ^{ba} | 2.21 ^{ba} | 2.44 ^{ba} | 2.65 ^a | 2.21 ^{ba} | 2.09 ^b | 2.21 ^{ba} | 2.38 ^{ba} | 2.11 ^b |
| AIA% | 2.02 ^a | 1.20 ^b | 1.80 ^a | 1.65 ^a | 2.10 ^a | 2.03 ^a | 1.84 ^a | 1.70 ^a | 1.81 ^a | 1.76 ^a |
| NDF% | 73.69 | 69.59 | 68.43 | 68.02 | 68.97 | 68.64 | 70.90 | 68.16 | 69.75 | 68.98 |
| ADF% | 56.36 | 55.28 | 53.84 | 51.87 | 51.61 | 53.01 | 54.57 | 55.89 | 55.47 | 52.14 |
| HC% | 17.33 | 14.32 | 14.59 | 16.16 | 17.36 | 15.64 | 16.32 | 12.28 | 14.28 | 16.85 |

a,b,c,d- Means bearing different superscripts in a column differ significantly ($*p < 0.05$)

DM=Dry matter, OM=Organic matter, CP= Crude protein, EE= Ether Extract, AIA= Acid insoluble ash, NDF= Neutral detergent fiber, ADF= Acid detergent fiber, HC= Hemicelluloses.

Rumen metabolites

Analysis of rumen metabolites revealed an increase ($P < 0.01$) in level of ammonia (NH_3), total nitrogen (total-N), non protein nitrogen (NPN-N) and trichloroacetic acid precipitable nitrogen (TCA-N) with increasing the level of urea [Table-2]. The rise in different nitrogen fractions was due to treatment of soluble nitrogen source in the form of urea. However, NPN-N was found almost double than TCA-N because incorporation of nitrogen from non protein nitrogen sources as urea.

Similar consistent results were also reported on urea treated rice straw [8]. Volatile fatty acids (VFA) were significantly ($P < 0.05$) higher in the treatment groups compared to control but comparable among treatments. The general increase in VFA due to the treatments was probably a reflection of the improvements in the fermentation rate, which resulted due to increased digestibility of DM. The optimum effect of urea-moisture treatment of SCB was observed for 3.5% urea and 40% moisture, predominantly for digestibility and VFA production.

Table-2 Effect of urea and moisture treatment of sugarcane bagasse on rumen metabolites and in vitro gas production parameters

| Moisture % | 0 | 40 | | | 50 | | | 60 | | | Significance | | |
|---|--------------------|---------------------|----------------------|---------------------|----------------------|----------------------|---------------------|----------------------|---------------------|---------------------|--------------|----|-------|
| Urea % | 0 | 3.5 | 4.0 | 4.5 | 3.5 | 4.0 | 4.5 | 3.5 | 4.0 | 4.5 | | | |
| Groups | Control | T1 | T2 | T3 | T4 | T5 | T6 | T7 | T8 | T9 | U | M | U x M |
| NH_3 mg/dl | 15.47 ^b | 18.20 ^{ba} | 18.83 ^{ba} | 21.21 ^a | 17.99 ^{ba} | 18.27 ^{ba} | 20.30 ^{ba} | 19.18 ^{ba} | 19.67 ^{ba} | 20.44 ^{ba} | ns | ns | ns |
| Total-N mg/dl | 18.27 ^a | 32.97 ^{dc} | 37.31 ^{ba} | 40.60 ^a | 33.18 ^{dc} | 35.56 ^{bc} | 38.71 ^{ba} | 31.36 ^d | 36.19 ^{bc} | 39.83 ^a | * | ns | ns |
| NPN-N mg/dl | 11.34 ^d | 21.91 ^{bc} | 24.99 ^{ab} | 26.95 ^a | 22.96 ^{abc} | 23.87 ^{ab} | 25.97 ^{ab} | 19.32 ^c | 23.52 ^{ab} | 26.81 ^a | * | ns | ns |
| TCA-N mg/dl | 9.93 ^d | 11.06 ^{bc} | 12.32 ^{bac} | 13.65 ^a | 12.22 ^c | 11.69 ^{bac} | 12.74 ^{ba} | 12.04 ^{bac} | 12.67 ^{ba} | 13.02 ^{ba} | * | ns | ns |
| TVFA meq/dl | 6.56 ^c | 10.99 ^a | 9.91 ^{ba} | 8.81 ^b | 10.80 ^{ba} | 10.20 ^{ba} | 10.31 ^{ba} | 10.85 ^{ba} | 9.11 ^{ba} | 9.65 ^{ba} | * | ns | ns |
| In vitro gas production parameters | | | | | | | | | | | | | |
| IVOMD % | 44.25 ^b | 56.50 ^a | 52.86 ^{ba} | 51.35 ^{ba} | 56.04 ^a | 51.46 ^{ba} | 51.55 ^{ba} | 52.78 ^{ba} | 51.38 ^{ba} | 49.72 ^{ba} | ns | ns | ns |
| IVDMD % | 39.64 ^b | 51.51 ^a | 50.46 ^a | 48.61 ^{ba} | 51.28 ^a | 46.82 ^{ba} | 47.89 ^{ba} | 48.02 ^{ba} | 49.09 ^{ba} | 46.00 ^{ba} | ns | ns | ns |
| IVGP % | 5.50 ^b | 20.25 ^a | 17.75 ^a | 17.75 ^a | 16.75 ^a | 15.50 ^a | 14.75 ^a | 16.50 ^a | 15.50 ^a | 15.00 ^a | ns | ns | ns |
| ME(MJ/kgDM) | 10.44 ^c | 17.86 ^a | 17.96 ^a | 17.78 ^a | 18.12 ^a | 18.08 ^a | 18.39 ^a | 16.98 ^{ab} | 17.83 ^a | 17.98 ^a | ns | ns | ns |
| PF | 72.94 ^b | 204.57 ^a | 132.07 ^a | 187.41 ^a | 171.85 ^a | 161.92 ^a | 167.17 ^a | 179.48 ^a | 165.35 ^a | 171.74 ^a | ns | ns | ns |

U = effect of urea, M = effect of moisture, U x P = urea and moisture interaction.

a,b,c,d -Means bearing different superscripts in a column differ significantly * $p < 0.05$, ** $p < 0.01$, ns = Non-significant ($p > 0.05$).

NPN-N= non protein nitrogen, TCA-N= Trichloroacetic acid precipitable nitrogen, TVFA= Total volatile fatty acid

IVDMD = *In vitro* dry matter digestibility, IVOMD = *In vitro* organic matter digestibility, ME=Metabolisable energy, PF=Partitioning factor,

ME (MJ/kg DM) = $2.20 + 0.136 \times \text{gas produced (ml/200 mg DM)} + 0.0057 \times \text{CP (g/kg DM)} + 0.0029 \times \text{EE}^2 \text{ (g/kg DM)}$

PF= Truly digestible organic matter (TDOM)/ gas volume produced.

In vitro substrate degradability and gas production

There was significant ($P < 0.05$) improvement in *in vitro* parameters as compare to untreated SCB but the rises was similar amongst the treatment group [Table-2]. The IVDMD and IVOMD of treatment groups T1 and T4 being higher ($P < 0.05$) than control while other groups have acquired a mediocre values. This can be due to the urea (ammonia) treatment causes partial break down of the bond between

the lignin and other cell wall components that lead rumen bacteria to degrade fibrous material in the rumen [9]. Similarly, increased digestibility of dry and organic matter of treated straw was observed under *in vitro* conditions because of associative effect of urea with lime treatment in different combinations [10]. *In vitro* gas production was higher ($P < 0.05$) in all the treatment groups, which might be due to improved OM digestibility in respective groups. The gas production is

basically the result of fermentation of carbohydrates to volatile fatty acid. Similarly, significant differences for *in vitro* gas production were observed for three different strain of rice straw [11].

Conclusion

SCB treated with 3.5 % urea and 40% moisture for three weeks, enhanced CP and improves nutrient digestibility with positive impact on rumen metabolites and *in vitro* VFA and gas production. Treated SCB could be a best alternative to maintain the livestock during draught or shortage of feed resources.

Abbreviations

SCB: sugarcane bagasse; IVDMD: in vitro dry matter digestibility; IVOMD: in vitro organic matter digestibility; IVGP: in vitro gas production; VFA: volatile fatty acid

Author Contributions

PATEL V. R. planned and lookout the entire experiment including writing of manuscript. CHOUBEY M. helped in analytical process of substrate and rumen liquor. RAVAL A. P. helped in feeding of donor animal, collection of rumen liquor and writing of manuscript.

Conflict of Interest

Authors declare that they have no Conflict of Interest.

Acknowledge/ Funding resource

The experiment was funded by Director of Research, NAU, Navsari. Donor animals were maintained at Livestock Research Station, NAU, Navsari and laboratory facilities availed by Department of Animal Nutrition, College of Veterinary science & A. H., NAU, Navsari. Authors acknowledge the help rendered by NAU, Navsari.

Conflict of Interest: None declared

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