

In vitro gas production of urea-molasses treated rice (*Oryza sativa*) husk

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Abstract

The use of rice husk as a source of feedstuff for ruminants is limited by its high fibre content and low digestibility; however, this could be improved by treatment with urea and molasses. This study was conducted to assess the changes in the chemical composition and *in vitro* gas production of rice husk treated with urea-molasses solution. Samples of rice husk were sundried, milled, and treated with graded levels of urea-molasses solution prepared by mixing 2 kg of urea and 10 kg of molasses in 100 liters of water, resulting in a stock solution used to treat 100 kg of rice husk. The treatments were grouped as treatment 1 (T1 untreated), T2 (0.5% urea-molasses solution), T3 (1%), T4 (1.5%), and T5 (2.0%) based on the volume percentage of the urea-molasses solution applied. Data collected were analysed using ANOVA and mean separated by Duncan Multiple Range Test. The results obtained showed a significant ($P < 0.05$) variation in crude protein (%) 8.36(T₁) to 13.92(T₅), crude fibre 9.00(T₅) to 15.88(T₁), Ether extract 4.42(T₁) to 5.88(T₅), NDF 50.26(T₅) to 59.12(T₁), ADF 32.60(T₅) to 41.24(T₁) and ADL 22.72(T₅) to 30.32(T₁). Fermentation of the insoluble but degradable fraction (b, mL) improved with the increased inclusion of urea-molasses solution, with values ranging from 24.67 (T₁) to 36.67 (T₅). Gas volume production (mL) at 24hr of incubation was significantly ($P < 0.05$) affected by treatment. The estimated parameters varied significantly ($P < 0.05$). Estimated OMD (%) ranged from 55.72(T₁) to 70.84(T₅), ME (MJ/Kg DM) ranged from 8.29(T₁) to 10.84(T₅) and SCFA (mmol) varied from 0.688(T₁) to 1.007(T₅). Treatment of rice husk with urea-molasses solution, improved significantly the estimated OMD, ME and SCFA. Therefore, urea-molasses solution-treated rice husk may be fed as supplement for ruminants.

Keywords: Urea-molasses, *in vitro* gas production, rice husk, ruminant.



Production de gaz *in vitro* de balles de riz (*Oryza sativa*) traitées à l'urée-mélasse

Résumé

L'utilisation des balles de riz comme source d'aliment pour les ruminants est limitée par leur teneur élevée en fibres et leur faible digestibilité. Cependant, ces caractéristiques peuvent être améliorées par un traitement à l'urée et à la mélasse. Cette étude a été menée pour évaluer les changements dans la composition chimique et la production de gaz *in vitro* des balles de riz traitées avec une solution d'urée-mélasse. Des échantillons de balles de riz ont été séchés au soleil, broyés, puis traités avec différents niveaux d'une solution d'urée-mélasse préparée en mélangeant 2 kg d'urée et 10 kg de mélasse dans 100 litres d'eau, obtenant ainsi une solution mère utilisée pour traiter 100 kg de balles de riz. Les traitements ont été répartis comme suit : traitement 1 (T1, non traité), T2 (solution à 0,5 % d'urée-mélasse), T3 (1 %), T4 (1,5 %) et T5 (2,0 %), selon le pourcentage volumique de la solution appliquée. Les données collectées ont été analysées par ANOVA, et les moyennes comparées à l'aide du test de Duncan. Les résultats ont montré des variations significatives ($P < 0,05$) pour les paramètres suivants : protéines brutes (%) de 8,36 (T₁) à 13,92 (T₅), fibres brutes de 9,00 (T₅) à 15,88 (T₁), extrait étheré de 4,42 (T₁) à 5,88 (T₅), NDF de 50,26 (T₅) à 59,12 (T₁), ADF de 32,60 (T₅) à 41,24 (T₁) et ADL de 22,72 (T₅) à 30,32 (T₁). La fermentation de la fraction insoluble mais dégradables (b, mL) s'est améliorée avec l'augmentation de l'inclusion de la solution d'urée-mélasse, avec des valeurs allant de 24,67 (T₁) à 36,67 (T₅). La production de gaz (mL) après 24 heures d'incubation a été significativement affectée ($P < 0,05$) par le traitement. Les paramètres estimés ont également varié significativement ($P < 0,05$) : la digestibilité *in vitro* de la matière organique

(OMD, %) est passée de 55,72 (T1) à 70,84 (T5), l'énergie métabolisable (ME, MJ/kg MS) de 8,29 (T1) à 10,84 (T5) et les acides gras volatils (SCFA, mmol) de 0,688 (T1) à 1,007 (T5). Le traitement des balles de riz avec une solution d'urée-mélasses a significativement amélioré l'OMD, la ME et les SCFA estimés. Ainsi, les balles de riz traitées à l'urée-mélasses peuvent être utilisées comme complément alimentaire pour les ruminants.

Mots-clés : Urée-mélasses, production de gaz *in vitro*, balles de riz, ruminants.

Introduction

In the developing countries of the world, ruminants depend on year-round grazing on natural pastures, household wastes, cut grass and crop residues (Akinfemi and Ogunwole, 2012). In the Northern part of Nigeria, farmers face seasonal dry periods in which the pastures decrease in quality and quantity with evidence decrease in crude protein and available energy (Akinfemi *et al.* 2012).

Feeding only crop residues to ruminants during the dry periods does not provide enough nutrients that will ensure high productivity because of its low nutrient content, associated with the high lignin content. The value of rice husk as a feedstock for ruminants is affected by its high level of lignification and silicification, low nitrogen content, as well as the slow and limited ruminal degradation of the carbohydrate content (Van Soest, 2006). This is probably responsible for the movement of herdsmen and their livestock towards the Southern parts of the country, which attract certain difficulties such as undue exposure to cold, wild animals and frequent clashes with crop farmers. An attempt to resolve these conflicts between herdsmen and crop farmers led to the establishment of farm settlements in the Southern part of Nigeria to cater for the forage needs of the Northern livestock farmers. However, this has generated some tension and disaffection between the two geopolitical zones necessitating a better alternative.

The Northern part of Nigeria produce large tonnage of grains with proportionate large quantities of crop residues generated. Rice is produced in almost all regions of Nigeria with leftovers after processing. The residues generated from rice processing are rice straw, husk and bran. Rice husks are the hard protecting coverings of rice grains. They are formed from hard materials including silica and lignin, to protect the seed during growing season.

Although, there is paucity of information on treatment of rice husk, however, any treatment that enhances the nutrient value of the straw will also improve the nutrient quality of the husk. Overcoming the intrinsic barriers to rumen microbial fermentation is a key to improving the use of crop residues for ruminants (Sarnklong *et al.*, 2010).

Where rice husk is fed to ruminants in Nigeria, it is usually untreated, this is especially so because livestock production is basically extensive system. The decision on which method of treatment to apply in improving the nutritional stations of rice husk is dependent on the farmer. Where urea is used to treat crop residue, it is usually without the addition of energy source; although, it may be combined with other energy feed resources in a complete feed mixture. Urea does not contain any energy, hence, the addition of molasses, which is a source of soluble carbohydrate.

The aim of this study is to treat rice husk with urea-molasses with the view of improving its nutrient contents and to assess changes in the chemical composition and *in vitro* digestibility.

Materials and methods

Preparation of Experimental Samples

Samples of rice husk were obtained from the Teaching and Research Farm of Yaba College of Technology, Lagos, located at coordinates 6°37'1.9"N, 3°19'12.0"E (6.617194°N, 3.320000°E). Feed-grade urea was dissolved in water at a concentration of 0.7 M (moles per liter) as described by Alhassan and Aliyu (1991). This urea solution was then mixed with molasses to prepare stock solutions containing molasses at concentrations of 0.5%, 1.0%, 1.5%, and 2.0% (w/v), corresponding to treatments T2, T3, T4, and T5, respectively and were sprayed on 100g quadruplicate samples on DM bases. The urea-molasses treated samples were stored in airtight containers for one week before sun drying. Afterward, the samples were thoroughly mixed

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and sun dried. Samples designated for proximate composition and in vitro digestibility analyses were oven dried at 105°C for several hours until a constant weight was achieved.

Chemical Analysis

Nitrogen (N) content of the agricultural wastes was determined by the standard Kjeldhal method (Van Soest *et al.*, 1991) and the amount of crude protein was calculated (Nx6.25). Neutral detergent fiber (NDF), acid detergent fiber (ADF), acid detergent lignin (ADL), and crude fiber (CF) were also assessed using standard methods (Van Soest *et al.*, 1991).

In vitro Gas Production

Before the morning feed, rumen fluid was extracted from three West African dwarf female goats using an oesophageal suction tube. 40% concentration (40% maize, 10% wheat offal, 10% palm kernel cake, 20% groundnut cake, 5% soybean meal, 10% brewer's grain, 1% common salt, 3.75% oyster shell, and 0.25% fishmeal) and 60% Guinea grass were fed to the animals. Three batches of the 120ml calibrated syringes were incubated at 39°C. 30ml inoculums containing cheese cloth-strained rumen liquor and buffer (9.8g NaHCO₃ + 2.77g Na₂HPO₄ + 0.57gKCL + 0.47gNaCl + 0.12gMgSO₄.7H₂O + 0.16gCaCl₂.2H₂O in a ratio of 1:4 v/v) was added to 200mg sample in the syringe under continuous flushing with CO₂.

The gas production was measured at 3, 6, 9, 12, 15, 18, 21, and 24 hrs. After 24hrs of incubation, the amount of methane produced was estimated by introducing 4ml of 10M NaOH (Fievez *et al.*, 2005). The average volume of gas produced from the blanks was deducted from the total volume of gas produced. Fermentation characteristics were estimated using the equation $Y = a + b(1 - e^{-ct})$ (Flachowsky *et al.*, 1996). Qrskov and Mcdonald (1979) described this process as follows: Y = volume of gas produced at time't', a = intercept (gas produced from the soluble fraction), b = gas production rate constant for the insoluble fraction, (a + b) = final gas produced, C = gas production rate constant for the insoluble fraction (b), and t = incubation time. Menke and Steingass (1988) measured the amount of digestible energy (ME, MJ/Kg DM) and the percentage of organic matter that was digestible, and Getachew *et al.*

(2000) evaluated the amount of short chain fatty acids (SCFA) (1998).

$$ME \text{ (MJ/kg DM)} = 2.20 + 0.136 *Gv + 0.057 * CP + 0.0029 *CF$$

$$OMD = OMD = 14.88 + 0.88Gv + 0.45CP + 0.651XA$$

$$SCFA = 0.0239 *Gv - 0.0601$$

Where, Gv, CP, CF and XA are net gas production (ml/200mg DM), crude protein, crude fibre and ash of the incubated sample respectively.

Prediction of dry matter intake: The percent NDF was used to predict dry matter intake expressed as a percentage of body weight according to Schroeder (1994). The formula used for calculation was: PDMI (as % of body weight) = $120 \div \% \text{ NDF}$ where, PDMI is the predicted dry matter intake, and %NDF is the percentage content of neutral detergent fibre in forage

Statistical Analysis

Data obtained were subjected to analysis of variance (ANOVA) and where significant difference occurred means were separated by Duncan method (Duncan, 1955) using Statistical Analysis System (SAS) package and significance was declared when $p \leq 0.05$.

Results and discussion

The result obtained for chemical composition of urea-molasses treated rice husk is shown in Table 1. The crude protein content of rice husk increased progressively as urea-molasses treatment augmented from 0.5 (T2) to 2% (T5). The increase in the crude protein content may be due to urea-molasses treatment of rice husk. Researchers (Fall, 1988; Aregheroe, 2005) obtained CP increase in urea treated corn stover from 6 to 15% and an increase of 0 to 7% in urea treated forage. Others (Golmahi *et al.*, 2006) found a small increment (4%) in CP of barley treated with urea at 0 and 4%. According to Flachowsky *et al.* (1996), the nitrogen incorporated during treatment is readily available for use by rumen microbes as confirmed by the high rumen ammonia levels on urea treated stover.

Table 1: Chemical composition (g/100gDM) of urea-molasses treated rice husk

	T ₁	T ₂	T ₃	T ₄	T ₅	SEM
Crude protein	8.36 ^e	10.51 ^d	12.36 ^c	13.39 ^b	13.92 ^a	0.16
Crude fibre	15.88 ^a	12.18 ^b	10.12 ^c	9.02 ^d	9.00 ^e	0.24
Ether extract	4.42 ^e	4.68 ^d	5.39 ^c	5.61 ^b	5.88 ^a	0.08
Ash	14.61 ^e	14.74 ^d	15.41 ^c	15.71 ^b	15.96 ^a	0.20
Nitrogen free extract	56.73 ^b	57.89 ^a	56.72 ^b	56.27 ^c	55.24 ^d	0.15
Hemicellulose	17.88 ^c	18.93 ^b	17.40 ^d	15.25 ^e	19.71 ^a	0.20
Cellulose	10.92 ^c	9.79 ^d	10.70 ^c	11.00 ^a	9.28 ^e	0.15
Neutral detergent fibre	59.12 ^a	56.95 ^b	54.21 ^c	51.71 ^d	50.26 ^e	0.20
Acid detergent fibre	41.24 ^a	38.02 ^b	36.81 ^c	35.01 ^d	32.60 ^e	0.25
Acid detergent lignin	30.32 ^a	28.23 ^b	26.11 ^c	24.01 ^d	22.72 ^e	0.15

^{a-e} Means on the same row with different superscripts are significantly different ($p < 0.05$)

Urea is a non-protein nitrogen that does not contain any energy, therefore, the addition of molasses to the urea treated substrate. Fermentable energy supplements such as molasses may further increase the efficiency of incorporation of urea nitrogen to microbial protein in the rumen. The use of poor quality roughages by ruminants cannot be expected to improve by supplementation with readily fermentable carbohydrates, in the absence of rumen degradable nitrogen (Castrillo *et al.*, 1995).

In this experiment, treatment effect as affected by the CF, NDF and ADL is significant ($p < 0.05$). The NDF, ADF and ADL decreased with increase in inclusion of urea. The Hemicellulose (%) content decrease in T₃ (17.40), T₄ (15.25) while cellulose content decrease in all the treated samples. Decrease in NDF content in all treated rice husk may be the result of hemicellulose solubilisation (Sundstol and Coxworth, 1984). Earlier studies (Ramirez *et al.*, 2007) reported reduction of NDF in urea treated crop residues and grass hays. Additionally, Rodriguez *et al.* (2002) found that *Brachiaria humidicola* hay ammoniated with a urea solution at 6% decreased the NDF compared with the untreated. Others (Cameque *et al.*, 1988) added that NDF concentration was reduced in barley straw treated with urea at 6%. Elsewhere (Aregheore, 2005; Oji *et al.*, 2007) observed reduction cell wall contents of urea treated maize residues at 7%. Reduction in fiber components or cell wall carbohydrates, such as NDF, ADF, and ADL, following urea treatment of crop residues generally has positive implications for livestock

feeding. Urea treatment breaks down lignin and hemicellulose bonds, making cellulose and other carbohydrates more accessible to rumen microbes, thereby enhancing digestibility and nutrient availability (Abdulazeez *et al.*, 2021; Alli-Balogun, *et al.*, 2018). This improved digestibility leads to higher voluntary feed intake, better nutrient utilization, and increased animal performance, including higher weight gains and improved body condition (Abdulazeez *et al.*, 2021; Alli-Balogun, *et al.*, 2018; Khanal, *et al.*, 1999). For example, studies have reported that animals fed urea-treated crop residues consumed more feed and had higher live weight gains compared to those fed untreated residues Khanal, *et al.*, 1999; Alli-Balogun, *et al.*, 2018). However, while the reduction in fiber enhances digestibility, it is important to maintain sufficient effective fiber in the diet to support proper rumen function and chewing activity (IAEA (n.d.)). Overall, the reduction in cell wall carbohydrates through urea treatment is considered advantageous for ruminant nutrition, especially when using low-quality crop residues as a major feed resource

The result of the *in vitro* gas production and fermentation characteristics is presented in Table 2. The effect on the treated rice husk is significant ($p < 0.05$) with volume of gas increasing as with the increased inclusion of urea-molasses. Gas production is closely associated with digestibility, the more the gas produced the better the digestibility. The amount of gas to be produced during fermentation may depend on the nature and level of fibre, presence of secondary metabolites (Babayemi *et al.*, 2004a) and potency

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of the rumen liquor for incubation. If the donor animal from which the rumen fluid for incubation was collected met the nutrient requirement, the potential gas generation of a feedstuff might be achieved (Babayemi, 2007). The amount of gas produced generally relies on the type of degradable carbohydrate, which affects how much gas is created (Demeyer and Van Nevel, 1975; Blummel and Becker, 1997). The volume of gas produced in this study progresses, and there is a chance that more gas will be produced after the initial 24 hours have passed. The increase in microbial proliferation brought on by the addition of urea to the rumen may be the cause of the treated sample's large volume of gas production.

Methane (mL/200mgDM) production ranged from 4.00 to 14 among the samples investigated

the least and highest being from T₅ and T₁ respectively. Feedstuffs characterised by high capacity for gas production are not usually observed to show high methane production. This seems not to be the case in this study. Methane production indicates an energy loss to the ruminant and many tropical feedstuffs have been implicated to increase methanogenesis (Babayemi *et al.*, 2004a; Babayemi and Bamikole, 2006a; Babayemi and Bamikole, 2006b) as an integrated part of carbohydrate metabolism (Demeyer and Van Nevel, 1975). High methane production observed in the untreated sample T₁, may be due to the higher fibre content compared with the treated sample, leading to slower fermentation.

Table 2: *In Vitro* gas production (mL/200gDM) and gas production characteristics of urea-molasses treated rice husk

Incubation Time (h)	T ₁	T ₂	T ₃	T ₄	T ₅	SEM
3	6.67 ^d	18.00 ^c	18.0 ^c	23.66 ^b	28.00 ^a	0.21
6	10.0 ^c	18 ^d	20 ^c	25.67 ^b	30.00 ^a	0.31
9	12.0 ^e	20.0 ^e	20.0 ^c	27.0 ^b	30.00 ^a	0.24
12	14.67 ^e	21.67 ^d	22.00 ^c	28.00 ^b	31.00 ^a	0.66
15	16.00 ^d	22.3 ^b	24.67 ^c	29.00 ^{ab}	31.67 ^a	0.88
18	18.07 ^d	26.67 ^{bc}	22.67 ^{cd}	30.00 ^b	34.33 ^a	0.75
21	19.33 ^d	28.0 ^c	35.00 ^b	37.00 ^{ab}	39.67 ^a	0.59
24	31.33 ^c	37.33 ^{bc}	39.33 ^{ab}	41.00 ^{ab}	44.67 ^a	1.13
CH ₄	14.00 ^a	10.00 ^b	7.00 ^c	6.00 ^c	4.00 ^d	0.13
Gas Production Characteristics						
b (mL)	24.67 ^c	28.07 ^{bc}	32.00 ^{ab}	34.33 ^{ab}	36.67 ^a	0.13
c (mL/h)	0.00071 ^e	0.0016 ^d	0.0028 ^c	0.0034 ^b	0.0066 ^a	0.00

^{a-e} Means on the same row with different superscripts are significantly different (p<0.05)

Estimated metabolizable energy (ME), organic matter digestibility (OMD), short chain fatty acid (SCFA), and potential dry matter intake (PDMI) are presented in Table 3. The value for the ME, OMD, SCFA and PDMI ranged from 8.29 (T₁) to 10.84 (T₅), 55.73 (T₁) to 70.84 (T₅), 0.688 (T₁) to

1.007 (T₅) and 2.02 (T₁) to 2.39 (T₅). Treatment effect as affected by the estimated parameters is significant (p<0.05). The value obtained in the present study was higher than those reported in literature for agro-industrial by-product wastes (Aregheore and Abdulrazak, 2005).

Table 3: Estimated metabolizable energy (ME), organic matter digestibility (OMD), short chain fatty acid (SCFA) and potential dry matter intake.

Parameter	T ₁	T ₂	T ₃	T ₄	T ₅	SEM
ME (MJ/KgDM)	8.29 ^c	9.44 ^{bc}	9.82 ^{ab}	10.13 ^{ab}	10.84 ^a	0.22
OMD (%)	55.73 ^c	62.06 ^b	65.09 ^b	67.21 ^{ab}	70.84 ^a	1.0
SCFA (µmol)	0.688 ^c	0.832 ^{bc}	0.880 ^{ab}	0.918 ^{ab}	1.007 ^a	0.03
PDMI (%BW)	2.02 ^e	2.11 ^d	2.21 ^c	2.39 ^a	2.32 ^b	0.00

^{a-c} Means on the same row with different superscripts are significantly different ($p < 0.05$). ME- Metabolisable energy, OMD-Organic matter digestibility, SCFA- Short chain fatty acid PDMI-Potential dry matter intake

Menke and Steingass (1988) reported a strong correlation between ME values measured *in vivo* and predicted from 24h *in vitro* gas production and chemical composition of feed. The *in vitro* gas production method has also been extensively employed to evaluate the energy value of several classes of feed (Aiple *et al.*, 1996; Getachew *et al.*, 1998; Getachew *et al.*, 2002). Krishnamodthy *et al.* (1995) also suggested that the *in vitro* gas production technique should be considered for estimating ME in tropical feedstuffs, because evaluation of ME by other techniques requires labour, cost, time and complexity.

High digestibility of organic matter were observed in the treated samples. This implies that the microbes in the rumen and animal have high nutrient uptake, also, the treatment affect curtailed the lignified barrier.

Short chain fatty acid, is the available energy and higher values were reported in the treated compared with the untreated. The higher SCFA predicted for T₂ to T₅ were obviously better than the untreated. This might be as a result of the treated material producing more. The creation of gas from various feed classes when incubated *in vitro* in buffered rumen fluid (Blummel and Orskov, 1993) is closely related to the synthesis of SCFA, which was based on carbohydrate fermentation. Others (Getachew *et al.*, 2002) reported a strong correlation between SCFA and gas production *in vitro*; this correlation was utilized to calculate SCFA production from gas values, which is an indicator of the animal's energy availability. The treated samples had

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greater PDMI values, which varied significantly ($p < 0.05$).

The huge fermentation of the insoluble but degradable fraction (b, ml) was observed in urea-molasses treated husk, possibly influenced by the carbohydrate fractions readily available to the microbial population (Chumpawade *et al.*, 2007). In comparison to the control (T₁), treatments T₂, T₃, T₄ and T₅ was found to have a higher potential for microbial breakdown, as indicated by the high value of the parameter (b, mL): T₂ (28.07), T₃ (32.0), T₄ (34.33) and T₅ (36.67) which represents the fermentable percentage of insoluble material. This implies that ruminants may benefit from treatment T₂, T₃, T₄ and T₅'s longer-lasting energy source. Furthermore, treatments T₂-T₅ had a greater gas production rate constant (c), suggesting a quicker rate of fermentation. The increased total gas output seen during the incubation phase may be a result of this quick breakdown of insoluble particles.

Higher values were observed in the urea-molasses treated samples T₂, T₃, T₄ and T₅, this suggests potential for high feed intake and subsequent high performance.

Conclusion

From the results obtained in this study, there was a remarkable improvement in the estimated ME, OMD and SCFA. Treatment effect on the proximate composition was significant indicating that urea-molasses treatment could be applied to upgrade low nutrient content feedstuffs.

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