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CHEMICAL COMPOSITION AND SILAGE QUALITY OF UREA, MOLASSES, AND UREA AND MOLASSES ENSILED SOYBEAN HUSK

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Abstract: The study evaluated the effect of additives treatment on the chemical composition and silage quality of soybean husk in a 28-day experiment, using completely randomised design. Soybean husk was chopped into 1-3 cm length and ensiled in a laboratory silo. There were four treatments consisting of the control (no additive treatment; T1), urea treatment (T2), molasses treatment (T3) and urea and molasses treatment (T4). The colour of the silages was goldenrod, dark goldenrod, peru and burly wood for T1, T2, T3 and T4 respectively. Except for T2 which had a pungent smell, silage smell was generally pleasant. All the silages had firm texture. Silage temperature, ether extract and propionic acid were (P>0.05) not affected by treatments. Silage dry matter and ammonia-nitrogen were higher (P<0.05) in T2 than in other treatments. Silage organic matter, non-fibre carbohydrates, neutral detergent fibre, acid detergent fibre, acid detergent lignin, hemicellulose, cellulose and butyric acid were higher P<0.05) in the control relative to the additive treatments. Crude protein of the silages was lowest and highest (P<0.05) in T1 and T4 respectively. Silage pH was lower in T3 than in other treatments. Acetic and lactic acids of the silages were affected by additives treatment, and increased in the order: T1 < T2 < T3 < T4 (P<0.05). In conclusion, additives treatment enhanced the nutritive and fermentation qualities of soybean husk. However, urea and molasses treatment produced the best results.

Key words: additives, ensiling, fermentation quality, nutritive value, soybean husk

Introduction

Insufficient availability and fluctuating quantity and quality feed is one of the major prevalent constraints to livestock production (Olafadehan and Adewumi, 2009). This problem becomes exacerbated during the dry season when native pastures are senesced, fibrous and lignified, and have little or no nutritional value (Olafadehan et al., 2009). The scarcity of green fodder and escalating demand for conventional ingredients consumed by humans have led to the utilisation of non-competitive and non-conventional agricultural wastes in livestock feeding. Similarly, occasional scarcity and high cost of conventional feed ingredients have prompted researchers to employ different processing methods and technologies such as urea ammoniation (Olafadehan and Adebayo, 2016; Olafadehan and Okoye 2017; Olafadehan et al., 2017), urea and molasses treatment (Lunsin et al., 2018) and solid state fermentation (Anaso and Olafadehan, 2021*;* Olafadehan et al., 2021, 2023) to improve the feeding value of poor quality agricultural wastes. However, additive treatment of lignocellulosic materials to improve the nutritive value is an easier and cheaper technology for farmers' adoption than the solid state fermentation, which requires some level of proficiency and expertise to practice.

Presently, in Nigeria, agro-industrial by-products and crop residues are copiously available due to increasing expansion of agro-industrial activities and diversion of open grazing land to crop production in an attempt to feed the ever teeming human population (Olafadehan and Adebayo, 2016). However, they constitute nuisance and environmental hazards due to the problem of poor disposal. Most often than not, they are either heaped and left to decay or burnt. If well harnessed and processed, they could guarantee all-year round feed availability for ruminant stock. One of such agricultural wastes is soybean husk (SBH) obtained from processing of soybean (*Glycine max L.*) after harvesting and threshing. Soybean is one of the commonly consumed legumes worldwide, with 200 million metric tons produced per year (Lim et al., 2011). However, the inedible SBH (the pod that covers the seeds) which is removed during threshing and processing, represents a major disposal problem for soybean industries.

Unlike cowpea husk, which is commonly fed to ruminant stock in Nigeria, SBH is not presently used as a feed ingredient. Therefore, the husk is mostly heaped and sometimes burnt or left on the field, thereby constituting environmental hazards. The fibrous, poor quality SBH can be harnessed and enhanced by ensiling with additives, such as urea and molasses, to improve its nutritive value, ameliorate the problem of environmental pollution due to its disposal and circumvent the problem of dry season feeding of ruminant animals. Silage, anaerobically fermented, preserved substrates or plant materials, has now become an increasingly important source of animal feed in the tropics in both dry and rainy seasons (Pholsen et al., 2016). Its production is an efficient conservation technology to improve the feeding value and ensure adequate feed supply when required. Urea and molasses have been used as additives for nutritional fortification or enhancement of lignocellulosic materials in a process involving ensiling. Previous studies involving use of urea and/or molasses (Olafadehan and Adebayo, 2016;

Abera et al., 2108; Lunsin et al., 2018) to ensile crop residues showed improvement in the nutritive value.

It was hypothesized that additive treatment of ensiled SBH would enhance its silage quality and nutritive potential. This study was conducted to investigate the effect of urea, molasses, and urea and molasses treatment and ensiling of SBH on its silage quality and nutritive value.

Materials and Methods

Experimental site

The experiment was conducted at the University of Abuja Teaching and Research Farm, Federal Capital Territory, Nigeria. The site is at 456 m altitude and lies between latitude 8º 55' N and 9º 00' E and longitude 7º 00' N and 7º 05' E. It has a tropical climate with temperature and annual rainfall ranging from 25.8 to 42°C and 1100 to 1650 mm respectively.

Silage preparation

Soybean husk, obtained from farmers, was chopped into 2-3 cm length for ease of compaction during ensiling. There were four treatments with four replicates per treatment. In treatment 1 (no additive; T1), 100 L of water was sprayed and mixed on 100 kg dry matter (DM) basis of SBH. In treatment 2 (T2), 4 kg of urea was dissolved in 100 L water, and the solution was carefully sprinkled and mixed with 100 kg (DM basis) of SBH. In treatment 3 (T3), 10 L of molasses was dissolved in 100 L of water, and the solution was thoroughly mixed with 100 kg (DM basis) of SBH. In treatment 4 (T4), 4 kg of urea and 10 L of molasses were carefully dissolved in 100 L of water, and the solution was mixed with 100 kg DM of SBH. The treated SBH was placed in individual labelled polyethylene bags which were compressed to eliminate air and the mouths tightly sealed. The polyethylene bags were then placed inside a large drum (experimental silo), compacted to make it air tight, sealed tightly with polyethylene sheets and then covered with a heavy object placed on the lid to prevent aeration and allow anaerobic fermentation for 28 days.

Physical evaluation of silage quality

After the 28 days ensiling, silage colour, smell, texture and temperature were determined immediately. Silage temperature was determined by inserting laboratory thermometer. Colour was assessed by visual appraisal with the aid of a colour chart. The aroma or smell and texture assessment were by five trained panellists.

Chemical analyses and calculation

The DM of the silages was determined by drying the samples at 60°C in a forced air oven until a constant weight was achieved (AOAC, 1995). After drying, the samples were ground through a 1 mm screen (Wiley mill, Standard Model 3, Arthur H. Thomas Co., Philadelphia, PA) for chemical analyses. Samples of the silages were analysed for their proximate constituents in accordance with the procedures of AOAC (1995). Neutral detergent fibre (NDF) and acid detergent fibre (ADF) were analysed using the procedures of Van Soest et al. (1991). NDF was analysed using sodium sulphite and amylase, and expressed with residual ash. Acid detergent lignin (ADL) was determined by solubilisation of cellulose with sulphuric acid. Concentrations of hemicellulose and cellulose were calculated as the differences between NDF and ADF, and ADF and ADL respectively. Non-fibre carbohydrate (NFC) was calculated using the following formula, NFC = 100 – (CP + EE + ash + NDF) %. Silage fermentation parameters were determined by mixing another sample (20 g) with 180 mL sterile water which was suspended at 4°C overnight and then filtered through four layers of cheesecloth. The filtrate was used to measure pH, ammonia-nitrogen (NH_3-N) and organic acids. NH_3-N content was determined according to the method of Broderick and Kang (1980). Organic acids (acetic, propionic, butyric and lactic acids) were analysed using high-performance liquid chromatography according to the methods of Wang et al. (2019).

Statistical analysis

Data were subjected to analysis of variance in a completely randomized design using SPSS Base 23.0 (SPSS software products, USA). Duncan multiple range test of the same software was used to test the significance of the differences among the means at $P \leq 0.05$. The statistical model is:

$$Yij = \mu + ti + eij$$

where Yij = the general response to the specific parameter under investigation, μ = the general mean peculiar to each observation, *ti* = the fixed effect of the additive treatments on the observed parameters and *eij* = the random error term for each estimate.

Results and Discussion

Chemical composition of the additives enhanced soybean husk silage

Silage DM was lower (P<0.05) in T3 and T4 compared to T1 and T2, though T2 had the highest (P<0.05) value. The lower DM of T3 and T4 relative to the other treatments (Table 1) suggests higher extent of fermentation by providing soluble substrates from molasses and the effect of urea on cell wall content of the substrates (McDonald et al., 1991). The result, therefore, implies loss of soluble carbohydrates during fermentation for T3 and T4. This decreased in DM of T3 and T4 concurs with the findings of Pour et al. (2018), who observed lower DM for silages ensiled with molasses and urea-molasses additives relative to untreated and urea-treated silages. The highest DM content of T2 indicates reduced DM loss during ensiling. Organic matter and NFC were lower ($P<0.05$) in the additives-treated silages compared to the control. The decrease in OM and NFC contents of the additives-treated groups suggests the availability and utilisation of the two chemical components by certain microbes for their growth and enhanced fermentability of the substrates (Olafadehan et al., 2012), resulting in increased lactic acid production. Non-fibre carbohydrate supplies readily fermentable carbohydrates which are fermented into organic acids, particularly lactic acid, under conducive anaerobic condition. However, the lower OM and NFC of T2 relative to T3 and T4 indicates less fermentability of the OM and NFC contents of urea ensiled SBH compared to molasses, and urea and molasses treated SBH. Crude protein was highest in T4, followed by T2, then T3, and lowest in T1 ($P<0.05$). The increased CP content of the additives-treated SBH indicates that addition of urea (a non-protein nitrogen source) and molasses (an energy source) improved microbial proliferation during fermentation to produce microbial protein (Kung et al., 2000). Moreover, both urea and molasses have been used to improve the CP contents of silages, in consistence with previous reports (Wanapat et al., 2013; Norrapoke et al., 2018, 2022). The increase in the CP content of T2, T3 and T4 also suggests efficient fermentation, preservation and stability of the silages, which possibly arrested and hindered the activities of different types of bacteria from performing their activity, thus making them available and becoming part of the silage. However, the higher CP content of T2 than T3 indicates that urea, might have been utilised to produce protein by certain organism, in corroboration previous findings (Norrapoke et al., 2022). The greater CP of T4 suggests that urea and molasses combination reduced proteolysis during ensiling and thus enhanced CP level better than urea only. The crude protein content of the additives-treated silages met the critical threshold requirement of 7% recommended for small ruminants (NRC, 1981).

Fibre fractions, hemicellulose and cellulose were lower ($P<0.05$) in the treatment groups compared to the control group, implying enhanced structural carbohydrates degradation with additives treatment. The lower fibre fractions of the T2 than the control may be due the enhancement of the nitrogen content of the urea-ensiled SBH which perhaps resulted in release of ammonia that reacted with cell wall components and thus reduced the fibre fractions. Similar observations were made by Olafadehan et al. (2017) and Lunsin et al. (2018). The reduction in structural carbohydrates of T3 relative to the control suggests increased activity of certain fibre-degrading microbes during the ensiling (Olafadehan et al., 2012; Olafadehan and Adebayo, 2016). Molasses, a good source of readily fermentable, is likely to have furnished certain fibre-degrading microbes with energy to enhance the fibre fractions, hemicellulose and cellulose degradation and thus reduced their concentrations in the silage. However, the lowest structural carbohydrates of T3 indicates that urea and molasses additive enhanced fibre degradation more effectively to produce silage of better quality and nutritive value than either urea only or molasses only, in tandem with earlier findings (Kang et al., 2018; Lusin et al., 2018).

Table 1. Effect of additives treatment on chemical composition (% DM unless otherwise stated) of soybean husk silage

Item	Treatment				SEM
	T1	T2	T3	T4	
Dry matter (% fresh matter)	31.5^{b}	32.7^{a}	28.3^{c}	27.7^{c}	3.89
Organic matter	96.9^{a}	94.8^{b}	92.9^{b}	91.3^{c}	1.66
Crude protein	5.80^{d}	8.84^{b}	6.70^{c}	9.55^{a}	0.32
Ether Extract	2.20	2.40	2.50	2.30	0.19
Non-fibre carbohydrate	29.72^{a}	29.28^{b}	28.54^{c}	28.30^{c}	0.29
Neutral detergent fibre	59.08^{a}	54.28^{b}	54.96^{b}	51.15^{c}	1.09
Acid detergent fibre	45.61^{a}	42.23^{b}	42.19^{b}	40.51^{c}	1.23
Acid detergent lignin	10.77^{a}	10.59^{b}	10.34^{b}	10.49^{c}	0.09
Hemicellulose	10.08^{a}	9.05^{b}	8.72^{b}	6.94^{c}	0.12
Cellulose	33.84^{a}	29.64^{b}	26.9^{b}	24.02^{c}	3.02

Means in the same row without a common superscript letter are significantly different ($P<0.05$)
T1: untreated soybean husk silage; T2: urea-treated soybean husk silage; T3: molasses-treated soybean husk silage; T4: urea and molasses treated-soybean husk silage

Physical characteristics of additives enhanced soybean husk silage

The silage colour was golden rod, dark golden rod, peru and burly wood for T1, T2, T3 and T4 respectively (Table 2). The peru colour of T3 is similar to the burly wood colour of T4 but the dark goldenrod colour of T2 may be due to the addition of urea, which perhaps affected the smell also. The colour of the silages was in order because good silage usually preserves well the original colour of the ensiled substrates or materials. All the silages had pleasant smell except T2 silage that had pungent smell due to addition of urea, which usually generates ammonia, a choking and pungent gas, during fermentation. Therefore, the pungent smell of T2 silage cannot be attributed to spoilage. All the silages had a firm texture which was desirable and previously reported as the best texture for a good silage (Kung and Shaver, 2001). The temperature of the silages was not affected ($P>0.05$) by additives treatment. It is worthy to say that the temperature of the silages was below 28°C, indicating well preserved silages. Bolsen et al. (1996) reported that excessive heat production during ensiling can result in maillard or browning reaction which compromises silage quality and nutritive value due to reduced protein and fibre digestibility when fed.

Table 2. Physical characteristics of additives treated soybean husk silage

Parameter	Treatment				SEM
	T1	T2	T3	T4	
Colour	Goldenrod	Dark goldenrod	Peru	Burly wood	-
Smell	Pleasant	Pungent	Pleasant	Pleasant fruity	-
Texture	Firm	Firm	Firm	Firm	-
Temperature (℃)	26	25.8	27.5	27.0	0.91

Means in the same row without a common superscript letter are significantly different (P<0.05)
T1: untreated soybean husk silage; T2: urea-treated soybean husk silage; T3: molasses-treated soybean husk silage; T4: urea and molasses treated-soybean husk silage

Silage fermentation quality

The preservation of ensiled substrates/materials depends on the adequate acid production to arrest activity of undesirable microorganisms under anaerobic conditions. pH is a critical index of the extent of silage fermentation and quality, and a low pH ensures better aerobic stability, and inhibits further fermentation and development of undesirable aerobic fungi, particularly yeast and mould, which cause aerobic deterioration of silage. In the present study, all the silages (Table 3) had either below or the benchmark pH 4.20 for well-fermented high moisture silage (McDonald et al., 1991). Urea treatment increased (P<0.05) silage pH compared to control and other additive treatments. However, molasses-treated and urea-treated silages had the lowest and the highest pH respectively (P<0.05). Whereas urea buffers the decrease in silage pH, molasses enhances the pH. The highest pH of T2 is obviously the result of hydrolysis of urea by enzyme urease to ammonia, which possibly improved the buffering capacity of the silage and bacterial activity. The lowest pH of T3 can be attributed to the treatment with molasses, a sugar-rich ingredient, which is commonly used to upgrade the water-soluble carbohydrates content of poor quality, fibrous substrates. Molasses has been used to enhance fermentation quality and nutritive value of silage by enhancing the supply of fermentable carbohydrates for improved growth of lactic acid bacteria (LAB) (Li et al., 2010). Though the silage microbes were not monitored in the current study, it, however, appears plausible to infer that the decreased pH values due to the increased acidity of both T3 and T4 inhibited the less acid-tolerant bacteria, like Clostridium and Enterobacter, which perhaps consequently reduced undesirable fermentation and proteolysis of the silages.

Though NH_3-N (as g/kg of total N) was highest (P<0.05) in T2 compared to other treatments, the values of the silages were below the threshold value of 100 g/kg of total N that indicates extensive proteolysis during ensiling (McDonald et al., 1991). Generally, high concentration of NH_3-N (12–15% of total N) in silages indicates highly degraded, ensiled substrate proteins due to increased number and activities of Enterobacter or Clostridia (Kumar and Singh, 1984). Clostridia usually produce NH_3-N from decomposed protein in silage materials. Since silage with NH_3-N level of less than 70 g/kg total N has been reported as excellent (Lima et al., 2010), the silages in the current study can be said to be excellent and of good quality. The pronounced NH_3-N (g/kg total N) of T2 silage was due to the presence of urea, which on hydrolysis releases NH_3.

Similarly, the organic acid values of all the silages in this study were within or similar to the ranges for good-quality silages in which the values for lactic acid, acetic acid, propionic acid and butyric acids are 4–7%, 1–3%, <0.1% and 0% respectively (Kung, 2008). Acetic acid was higher ($P<0.05$) in the additive treatments relative to the control. However, among the additive treatments, it was highest in T4, followed by T3 and lowest in T2 ($P<0.05$). The higher acetic acid in the additives-treated silages, particularly molasses, and urea and molasses treatments, suggests the activity of heterofermentative LAB which perhaps increased aerobic stability and anti-fungal activity, thus decreasing proliferation and growth of undesirable spoilage microbes, and improving silage fermentation quality. Though not determined in the current study, the large amounts of acetic acid in the molasses, and urea and molasses based silages probably reduced the yeast count, and resulted in greater aerobic stability compared to the control and urea-treated silages. Propionic acid concentration is usually almost negligible (especially in drier silages) or in very low concentrations (<0.1%) in good, well-fermented silages (Kung et al., 2018). Propionic acid was not ($P>0.05$) affected by additives treatment. The generally low and unaffected propionic acid indicates well-fermented and preserved silages. Propionic acid, in addition with other organic acids such as sorbic, benzoic and acetic acids, has reported to improve aerobic stability of silage at feed out through direct inhibition of yeasts and moulds (Auerbach et al., 2012).

Butyric acid was lower ($P<0.05$) in the additive treatments than the control. However, the concentration was far below the critical threshold level at which it depresses feed intake by animals. The presence of butyric acid, an acid with strong, foul rancid-butter smell, in silage is undesirable because its production is an energy-waste metabolism, and concentration > 5 g/kg DM indicates substantial clostridial metabolic activity, large DM losses and poor energy recovery, which compromise feed intake and health of animals (McDonald et al., 1991; Muck, 2010). Clostridial silages indicate excessive proteolysis producing a putrid, fishy or ammonia-like odour (Kung et al., 2018), and also have a low level of energy and high soluble protein, which reduce feed intake when fed to animals (Muck, 2011). From the foregoing, the generally low butyric acid content is thus an indicator of reduced clostridial fermentation during ensiling.

Lactic acid was affected ($P<0.05$) in the order: T4 > T3 > T2 > T1. As previously explained, the higher lactic acid in the molasses-based silages is desirable as it suggests stoppage of bacterial activity and thus nutrient losses during the ensiling. The molasses in both T3 and T4 must have furnished LAB with soluble carbohydrates, which perhaps resulted in increased accumulation of lactic acid and subsequent reduction of the pH of the silages. The result suggests relative abundance of Lactobacillus and decreased Enterobacter because Lactobacillus, a common bacterium in silages, plays an important role in lactic acid accumulation and pH decline (Ni et al., 2018; Yan et al., 2019). However, the presence of Enterobacter is undesirable in silage because they may compete with LAB for nutrients and produce NH_3-N. The reduced lactic acid concentration of the molasses-based silages has some implications in ruminant nutrition. This is because addition of molasses contributed to high lactic acid contents and low pH during silage fermentation to produce high quality silages which when fed to ruminants may mitigate methane production. Generally, lactic acid is secondarily fermented in the rumen by lactate-utilising bacteria, such as *Megasphaera elsdenii*, *Selenomonas ruminantium*, *Fusobacterium necrophorum* and *Veillonella parvula*, which use hydrogen to convert lactic acid to propionate (Dawson et al., 1997; Russell and Wallace, 1997), thus providing alternative pathway for utilising hydrogen that could have otherwise been used for methanogenesis.

Table 3. Fermentation quality of additives treated soybean husk (g/kg DM)

Parameter	Treatment				SEM
	T1	T2	T3	T4	
pH	4.11[a]	4.24[a]	3.89[c]	4.02[b]	0.24
NH_3-N (g	42.80[d]	55.14[a]	44.94[c]	46.86[b]	0.69
Acetic acid	16.26[d]	19.23[c]	22.29[b]	25.28[a]	0.39
Propionic acid	0.004	0.004	0.003	0.003	0.00
Butyric acid	1.93[a]	1.80[b]	1.52[c]	1.21[d]	0.09
Lactic acid	52.22[d]	59.14[c]	67.24[b]	69.80[a]	1.46

Means in the same row without a common superscript letter are significantly different ($P<0.05$)
T1: untreated soybean husk silage; T2: urea-treated soybean husk silage; T3: molasses-treated soybean husk silage; T4: urea and molasses treated-soybean husk silage

Conclusion

Additives (urea, molasses, and urea and molasses) treatment of soybean husk improved the quality and nutritive value of emanating silages. However, molasses only, and urea and molasses treatments produced silages of superior quality relative to urea treatment only. It is, therefore, concluded that although soybean husk can be ensiled without any additives, additives treatment enhanced the silage quality with urea and molasses treatment producing silage of superior quality. Further research to evaluate the feeding value of urea and molasses treated soybean husk silage *in vitro* and *in vivo* should be conducted.

Hemijski sastav i kvalitet silaže sojine ljuske tretirane ureom, melasom, i ureom i melasom

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Rezime

U ovoj studiji je ispitivan efekat tretmana aditiva na hemijski sastav i kvalitet silaže sojine ljuske u 28-dnevnom eksperimentu, koristeći potpuno randomizovan dizajn. Sojina ljuska je iseckana na 1-3 cm dužine i silirana u laboratorijskom silosu. Ispitivanje se sastojalo od četiri tretmana - kontrola (bez aditivnog tretmana; T1), tretmana ureom (T2), tretmana melasom (T3) i tretmana ureom i melasom (T4). Boja silaže je bila zlatna, tamno zlatna, tamno narandžasta i narandžasta za T1, T2 T3 i T4 respektivno. Osim T2 koji je imao oštar miris, miris silaže je uglavnom bio prijatan. Sve silaže su imale čvrstu teksturu. Temperatura silaže, etarski ekstrakt i propionska kiselina (P>0,05) nisu bili pod uticajem tretmana. Suva materija silaže i amonijak-azot su bili veći (P<0,05) u T2 nego u drugim tretmanima. Organska materija silaže, nevlaknasti ugljeni hidrati, NDF, ADF, kiseli deterdžent lignin, hemiceluloza, celuloza i buterna kiselina bili su viši P<0,05) u kontroli u odnosu na tretmane aditiva. Sirovi protein silaže je bio najniži i najviši (P<0,05) u T1 i T4 respektivno. Vrednost pH silaže je bio niži u T3 nego u drugim tretmanima. Sirćetna i mlečna kiselina silaže su bile pod uticajem tretmana aditiva i povećavale su se po redosledu: T1 < T2 < T3 < T4 (P<0,05). U zaključku, tretman aditiva je poboljšao hranljive i fermentacione kvalitete sojine ljuske. Međutim, tretman ureom i melasom dao je najbolje rezultate.

Ključne reči: aditivi, siliranje, kvalitet fermentacije, hranljiva vrednost, sojina ljuska

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Conflict of interest
The authors declare no conflict of interest.

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