RESEARCH ARTICLE

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Evaluation of Urea and Molasses Treatment on The Nutritional Composition of Guinea Corn Stalk

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ABSTRACT

The majority of the world's population relies on livestock as source of protein whereas the success of livestock production is reliant on availability of quality feeds which has been challenged by competition impose by geometric growth in human population. This study assessed the effect of molasses and urea treatment on the physical quality, proximate and amino acid composition of guinea corn silage as a means to augment protein content in guinea corn silage feed stocks. Guinea corn stalks samples collected randomly on farmlands around Federal University Wukari, Nigeria. After pulverization was divided into two portions and one portion subjected to molasses and urea treatment while the other served as control. Effect of treatment was assessed by estimating and comparing the physical quality, proximate and amino acid composition of the treated and untreated sample using standard methods. The result showed that the mean pH value became more acidic after treatment from 6.12 to 5.24. While the moisture, lipid and crude protein content were significantly increased due to treatment (P <0.05). There was also a significant elevation in amino acid content due to urea and molasses treatment in 14 out of the 18 amino acids determined at 95% confidence limit (P<0.05). And the total amino acid content of the silage sample was improved from 43.80 g/100g to 62.27 g/100g. The nature of amino acids in the treated silage (test sample) were in the order of prevalence total amino acid with acidic side chain (TAAA)>total amino acid with basic side chain (TBAA)>total aromatic amino acid (TArAA)>total sulphur containing amino acid (TSAA). The most concentrated amino acid was glutamic acid, an important amino acid responsible in building the immune system.

Keywords: guinea corn stalks silage, improved nutritional content, urea treatment.

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I. Introduction

The majority of the world's population relies on livestock as a source of protein whereas the success of livestock production is reliant on quality feeds, pests, disease control, environmental factors and so on [1], [2].

Challenges of feed ingredients shortages like wheat, corn, soya beans and so on have been a continued one due to competitions imposed by the ever-increasing human population [3], [4]. In Nigeria, the outbreak of farmers and herders' clashes has worsened the situation and consequently led to food shortages [5].

A means to close up the gap between the ever-growing human populations and manage farmer-herder clash and the availability of food for humans and feed stuff for livestock production has been on the front burner.

A number of researchers and policy makers have suggested the establishment of ranches in place of open-grassing as a means to manage continuous clashes between farmers-herders which in the long run has threatened food security.

Managing livestock in both ranches and open grassing is equally challenged since plant protein like soya bean and beans are usually in short supply and expensive. Even non-plant protein like corn, millet and sorghums available for animal feeding has witness a great decline. Hence, the need for continuous effort to replace more costly protein additives in animal diets with cheaper and less competitive feeding resources, as a means of reducing cost of feeding.

Employing the use of by-products like sorghums, millet and maize stalks will consequently reduce the cost of animal feeding and competition [6]. The treatment of such less nutritive by-product with nutritive additives and silage making does enhance its nutritional content [7]. Silage is a feedstuff manufactured by the fermentation of a crop, forage or agricultural by product of generally greater than 50 % moisture content through a process called enlisting in a container (silo).

This study assessed the effect of urea and molasses treatment on the nutritional composition of guinea corn straw silage, with a view to utilize the product in augmenting nutrient deficiency in livestock feedstuffs.

II. Materials and Methods

Guinea corn stalks samples were collected randomly on a farmland located around Federal University Wukari, Nigeria with the geographical coordinate 7º84713′N and 9º4652′E. After collections, samples were chopped to about 2–3 cm lengths (for ease of compaction and consolidation for silage)

as described by the Food and Agricultural Organizations. Then separated into two portions (1 kg each) and labelled test and control samples respectively.

The test sample was subjected to treatment as follows: A pit of 36 cm length and 36 cm width was dogged and the wall of the pit was properly covered with black polythene nylon sack, 1 liter of molasses, 40 g of urea and 5 g of salt (NaCl) were added to the sample and mixed properly. The mixture was filled into a nylon sack with proper compactness to remove or displace air until the pit is filled. The final compaction was made after which the black polythene nylon sack was wrapped over the sample. Sand of 2 kg weight was rolled over the material and allowed for 21 days to ferment anaerobically [8].

At the end of 21 days, the fermentation was terminated, and the silage was opened and air dried before quality assessment. The assessed qualities characterized include color, odor, moisture, texture, and pH. Color chart was used to ascertain the color silage corresponds to. The odor and smell of the silage is relatively assessed as to whether nice, pleasant, or fruity [8].

A. Determination of Silage Sample pH

A pH meter (JENWAY 2000 Model) was used to determine the pH of a 1.0 g air dried silage sample (test sample) that was solubilized in 10.0 mL distilled water for 10 minutes [4]. The same procedure was replicated on the control sample.

B. Proximate Determinations

1) Moisture content

Moisture content was found by estimating the difference of the net weight and the steady weight obtained after oven drying.

2) Ash content

Ash content was found by estimating the difference of the net weight and the weight obtained after heating in a furnace at 550oC for 60 minutes [4],[9].

3) Lipid content

A pre-cleaned 250 cm^3 capacity round bottomed flask containing 200 cm^3 of petroleum ether (40–60°C) and some anti bumping granules fitted with soxhlet extraction unit was used to extract 20 g of the test silage enclosed in the thimble. The extraction was carried out for about 8 hours within the temperatures of 40°C to 60°C by means of adjustable heating mantle until the solvent refluxed at a steady rate. The lipid content was estimated after removing and oven drying the extracted silage to a constant weight at 70°C and reweighed and the percentage of extractible lipid calculated [10]. The treatment was replicated to obtain lipid content of control sample.

4) Fiber content

The determination was done by acid hydrolysis of two grams of the test sample of silage followed by the hydrolysis of the acid free residue obtained with standard sodium hydroxide solution. The basic free residue was further subjected to incineration in a muffle furnace after which the final residue was reweighed. And the percentage crude fiber content was then estimated [10]. This procedure was replicated on the control samples of silage.

5) Crude protein

The crude protein content was obtained using the standard method developed by Kjeldahl comprising of three steps; digestion, neutralization, and titration, adopted by the Association of Official Analytical Chemists method [9] and Omoniyi *et al*. [4]. Total nitrogen content of the silage was first calculated then the percentage of crude protein using the conversion factor of 6.25 which is equivalent to 0.16 g of nitrogen per gram of protein [10]. This procedure was repeated for the control sample.

6) Carbohydrate content

The carbohydrate content was calculated by subtracting the percentage aggregate of crude lipids, crude protein, crude fiber, moisture, and ash from 100% as shown in the equation below:

$$CHO\ (\%) = 100\% - (\%crude\ protein + \%crude\ lipid + \%crude\ fibre + \%ash + \%moisture) \quad (1)$$

C. Determination of Amino acid Content

The sample was dried at 70°C to a constant weight by means of an oven, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH amino acid analyzer [10].

1) Defatting sample

The sample was rid of its fat content by means of chloroform/methanol mixture (2:1) using soxhlet apparatus. About 4 g of the sample wrapped in an extraction thimble were extracted for 15 hours [11].

2) Hydrolysis of the sample

To 2g of the fat free sample in a glass ampoule, 7 ml of 6M HCl was introduced, and oxygen ousted by passing nitrogen into the ampoule (to avoid possible oxidation of some amino acids like methionine and cysteine during hydrolysis). The glass ampoule was then closed with the aid of bunsen burner flame before transferring into an oven pre-set at 105°C±5°C for 24 hours. The ampoule was allowed to cool before opening at the tip and the content was filtered to remove the humans. For tryptophan determination, another 2 g of the defatted test samples was separately hydrolyzed with 4.2 M sodium hydroxide for 22 hours and then neutralized to pH 7.0 with 6 M of hydrochloric acid.

3) Analysis

Sixty (60 µL) then dispensed into an ion exchange HPLC chromatography coupled with Applied PTH Amino Acid Analyzer (Model 120A) to separate, characterize, and quantify free acidic, neutral and basic amino acids of the hydrolysate Quantification was obtained by using external amino acid standards and the results were corrected for the recoveries. Tryptophan was determined using colorimetric method after alkali (NaOH) hydrolysis [11], [12]. All analyses were conducted in triplicate for both the test and same procedure was repeated for the control sample. Predicted protein efficiency ratio (P-PER) was determined using the equation described by Adeyeye [13].

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$$P - PER = -0.468 + 0.454\ (Leu) - 0.105(Tyr) \quad (2)$$

D. Statistical Analysis

All recorded and calculated data were analyzed statistically to test the levels of significance difference between the test and control sample. The means were compared by means of paired t-test using SPSS statistical analysis software set at 95% confidence limit (P<0.05).

III. Result And Discussion

A. Silage Quality

The physical attribute for the test sample (treated silage) revealed a dark brown color in contrast to light brown obtained in the control sample (untreated silage) as shown in Fig. 1. The test sample displayed a pleasant aroma implying enhanced palatability [14], while the control sampled an earthy aroma. The mean pH values of the test and control samples were 5.24 and 6.12 respectively. The acidic pH in both the test and control sample will inhibit the growth of clostridia and enterobacteria responsible for the degradation of amino acid resulting in silage with a poor palatability and lower nutritional value nevertheless, the 5.24 pH recorded in the test sample is slightly above 4.90 pH reported to inhibit the growth of acid tolerant clostridia [15].

Fig. 1. Guinea corn plant, stalk, treated guinea corn stalk silage and untreated guinea corn.

B. Proximate Compositions

The proximate composition of the test sample (treated silage) and control sample (untreated) guinea corn stalks in terms of moisture content, ash content, crude protein, crude fiber, lipid, and carbohydrate content reveals that both samples are enriched with the mentioned proximate constituents as shown in Fig. 2.

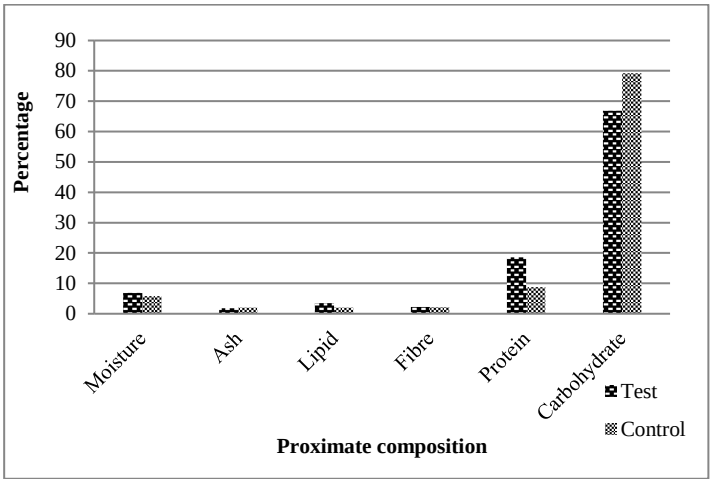

Fig. 2. Mean proximate composition of test (treated silage) and control sample (untreated silage).

1) Moisture Content

The mean moisture content of test and control samples were 6.92% and 5.79% respectively, where moisture content of the test sample was higher than that recorded in the control sample. These moisture contents were however less than the 8.94% moisture content recorded for maize corn silage reported from Abuja, Nigeria [16] implying greater stability of guinea corn silage compared to that of corn. Statistically, there is no significant difference (P<0.05) in moisture content of the test and control sample. The relatively higher moisture content of the test sample implies molasses and urea treatment lowers shelf life, since higher moisture content reduces shelf life [17].

2) Ash content

The test and control sample guinea corn stalk silage have their percentages ash content as 2.03% and 1.83% respectively. Which were relatively lower than the 2.43 % content recorded for sorghum samples in Lafia, Nigeria [18]. There was no significant difference in ash content between the test and control sample at 95% confidence limit (P>0.05), implying that molasses and urea treatment did not significantly affect the ash content. Ash content, been a measure of the overall quantity of mineral present in a giving sample suggest that the relatively high values of percentage ash content, the products would have substantial amount of mineral content [2].

3) Lipid content

The mean percentage Lipid content revealed 3.49% and 2.07% for the test and control sample respectively. Suggesting molasses and urea treatment significantly enhanced the lipid content at 95% confidence limit (P<0.05). However, the lipid content was less than the 4.50% reported for maize sample [19].

Lipid is also a source of energy. It protects organs, supports cell growth, keeps cholesterol and blood pressure under control. As well as helping the body to absorb vital nutrients [20].

4) Crude fiber content

Both the test and control sample recorded a sample have very low crude fiber of 2.15% and 2.21% respectively. These values were much less than the 11.00 % content recorded for cassava peel silage [13]. There's no significant

change on fiber content due to molasses and urea treatment at 95% confidence limit (p>0.05).

Dietary fiber content is composed of lignin and polysaccharides that cannot be broken down by human enzymes as well as indigestible starch [21].

5) Protein content

The crude protein content of the test sample 18.62% was more than twice the 8.72% content of the control sample as well as the 8.75% content recorded in maize plant [21]. There was significant difference in crude protein content at 95% confidence interval due to the effect of molasses and urea treatment (P<0.05). This suggests that urea and molasses treatment could serve as an alternative means of enhancing protein content of less nutritive feedstocks. Protein is an essential class of food needed for proper growth and replenishment of worn-out tissues.

6) Carbohydrate content

The carbohydrate content of the test and control samples were 66.92% and 79.25% respectively. There was a significant decrease in carbohydrate content due to molasses and urea treatment at 95% confidence level (P<0.05). The 66.92% carbohydrate content of the test sample was equally less than the 77.46% content recorded in maize [17]. The decrease in carbohydrate content during urea and molasses treatment as well as fermentation may be due to its utilization as energy resource during the anaerobic process.

7) Amino Acid Content

Both the test and control samples were found to be rich in all the 18 amino acids determined as shown in Table I. The mean amino acid composition of treated guinea corn stalks (Test sample) was Leucine 4.97 g/100g, Lysine 4.32 g/100g, Isoleucine 3.64 g/100g, Phenylalanine 3.99 g/100g, Tryptophan 0.59 g/100g, Valine 3.62 g/100g, Methionine 1.26 g/100g, Proline 3.36 g/100g, Arginine 4.33 g/100g, Tyrosine 2.95 g/100g, Histidine 1.53 g/100g, Cystine 1.14 g/100g, Alanine 3.47 g/100g, Glutamic acid 8.31 g/100g, Glycine 3.44 g/100g, Threonine 3.10 g/100g, Serine 2.87 g/100g and Aspartic acid 5.33 g/100g While the untreated guinea corn stalks (Control sample) was Leucine 3.80 g/100g, Lysine 3.02 g/100g, Isoleucine 3.05 g/100g, Phenylalanine 3.42 g/100g, Tryptophan 0.34 g/100g, Valine 2.77 g/100g, Methionine 0.82 g/100g, Proline 2.15 g/100g, Arginine 2.12 g/100g, Tyrosine 1.85 g/100g, Histidine 0.88 g/100g, Cystine 0.52 g/100g, Alanine 4.09 g/100g, Glutamic acid 4.29 g/100g, Glycine 2.35 g/100g, Threonine 2.38 g/100g, Serine 1.82 g/100g and Aspartic acid 3.85 g/100g respectively.

There was a significant elevation in amino acid content due to urea and molasses treatment in 14 out of the 18 amino acids determined at 95% confidence limit (P<0.05), with the exception of isoleucine, methionine and tyrosine (P>0.05).

The total amino acid content of test sample was 62.27 g/100g against the 43.80 g/100g found in the control sample. The contents were all less than the 90.85 g/100 g FAO/WHO standard requirement. Nevertheless, urea and molasses treatment made it possible for the content to elevate from 48.21% to 68.54 % of the FAO/WHO requirement [21]. The most abundant amino acid in both the test and control sample was glutamic acid 8.31 g/100g and 4.29 g/100g respectively. The least available amino acid was tryptophan 0.59 g/100g and 0.34 g/100g in the test and control samples respectively.

Glutamic acid, the most abundant amino acid in silage, plays an important role during biosynthesis of proteins. It is an excitatory neurotransmitter thereby aids improvement of memory and focus. Glutamic acid helps in building immune system, support prostrate health and detoxify the body [22].

The predicted protein efficiency ratio (P–PER) is one of the quality parameters used for protein evaluation [23], [24]; experimentally, within the scale of 0.0 for a very poor protein to a maximum possible of 4.0 [25], [26].

The 1.48 predicted protein efficiency ratio found in the test sample was higher than the 1.06 recorded in the control sample. However, both values were less than 50 % of the scale as well as the 1.67 ratio found in dung beetle silage [12], but greater than the 0.23 and 0.29 found in steeped and germinated guinea corn respectively [26].

TABLE I: MEAN AMINO ACID CONTENT OF TREATED AND UNTREATED GUINEA CORN STALK SILAGE

Amino acid	Test (g/100 g)	Control (g/100 g)	FAO/WHO Standard (g/100 g)	P-Value
Leucine	4.97±0.01	3.80±0.02	6.60	0.003
Lysine	4.32±0.04	3.02±0.00	5.80	0.012
Isoleucine	3.64±0.02	3.05±0.06	2.80	0.065*
Phenylalanine	3.95±0.05	3.42±0.07	6.30	0.012
Tryptophan	0.59±0.01	0.34±0.03	1.10	0.039
Valine	3.62±0.03	2.77±0.05	3.50	0.011
Methionine	1.26±0.02	0.82±0.03	2.50	0.058*
Proline	3.36±0.01	2.15±0.03	9.13	0.018
Arginine	4.33±0.04	2.12±0.08	8.00	0.026
Tyrosine	2.95±0.04	1.85±0.04	6.30	0.058*
Histidine	1.53±0.03	0.88±0.02	1.90	0.005
Cystine	1.14±0.00	0.52±0.05	2.50	0.036
Alanine	3.47±0.02	4.09±0.00	3.11	0.015
Glutamic acid	8.31±0.02	4.29±0.02	5.60	0.005
Glycine	3.44±0.02	2.58±0.04	7.12	0.007
Threonine	3.10±0.02	2.38±0.03	3.40	0.040
Serine	2.87±0.06	1.82±0.02	9.93	0.018
Aspartic acid	5.33±0.042	3.85±0.08	5.30	0.013
TOTAL	62.27	43.80	90.85	
P-PER	1.478	1.062	1.866	

*=(P>0.05) =No significant difference, P-PER=Predicted protein efficiency ratio, Treated (test sample), Untreated (control sample)

8) Essential amino acids

Essential amino acids cannot be synthesized by the body. As a result, they must be sourced through dietary intake. The total essential amino acid content in the test and control sample was 26.98 g/100g (43.32%) and 20.48 g/100g (46.75%) with the most prevalent amino acid being leucine (4.97 g/100g) in the test sample and (3.80 g/100g) in the control sample. The least available essential amino acid content was tryptophan 0.59 g/100g and 0.34 g/100g for the test and control sample respectively as presented in Fig. 3.

The percentage ratio of EAA to TAA in the test and control silage was 43.32 and 46.75%. These values were much above the 39 % considered to be suitable for a perfect protein nutrient for babies, 26 % for children and 11% for adult's base on FAO/WHO [21] stipulations. The inability of the body to have sufficient amount of essential amino acids could result in the degradation of body muscles [12], [27].

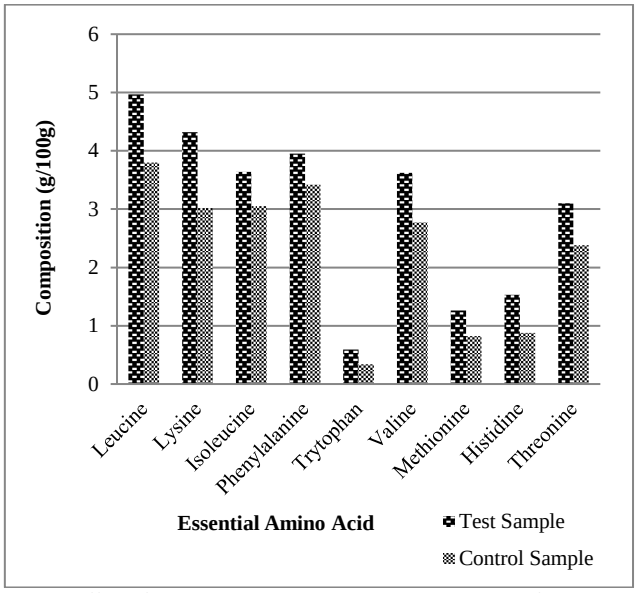

Fig. 3. Effect of processing on essential amino acid content of guinea corn silage.

9) Non-essential amino acids

These are amino acids that are synthesized by the human body in substantial amounts and hence they are non-essential. The total non-essential amino acid content in the test and control sample was 35.29 g/100g (56.67%) and 23.32 g/100g (53.24%) with the most prevalent amino acid being glutamic acid (8.31 g/100g) in the test sample and (4.29 g/100g) in the control sample as shown in Fig. 4.

However, these values were less than the 9.12 g/100g found in germinated guinea corn but that of the test sample was in agreement with the 8.20 g/100g found in steeped guinea corn [26]. The least available non-essential amino acid content was cysteine 1.14 g/100g and 0.52 g/100g in the test and control sample respectively.

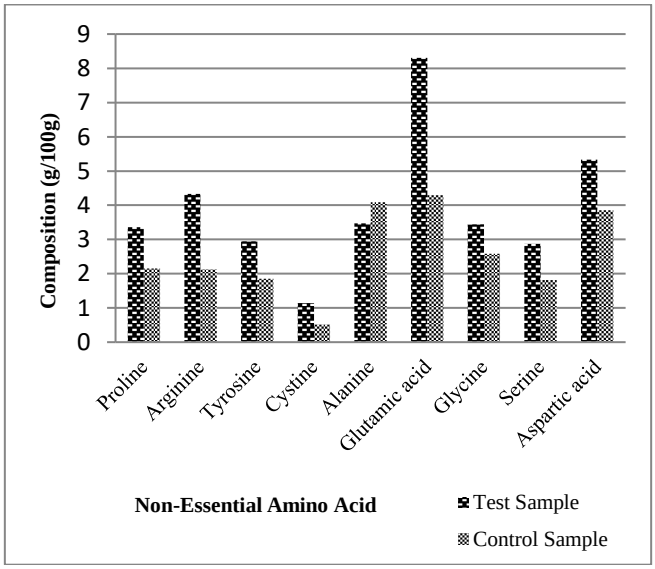

Fig. 4 Effect of processing on non-essential amino acid content of guinea corn silage.

10) Nature of Amino Acid in Silage

The prevalence of amino acids in the test and control sample were in the order total amino acid with acidic side chain (TAAA) > total amino acid with basic side chain (TBAA) > total aromatic amino acid (TArAA) > total sulfur containing amino acid (TSAA) as presented in Table II. This corresponds to the pattern of amino acid found fermented gamba grass silage incorporated with urea and molasses [28].

The two common amino acids with acidic side chain at neutral pH include aspartic acid and glutamic acid having carboxylic group with pKa low enough to lose proton thereby becoming negatively charged in the process. The total acidic amino acid (TAAA) of the test and control samples was 13.64 g/100g and 8.14 g/100g respectively. These values were all less than the TAAA of 23.93 g/100g, 18.14 g/100g and 22.78 g/100g found in catfish, dung beetle and crayfish respectively [12]. However, molasses and urea treatment increased the TAAA by 40.32% of its original level.

The common amino acids with basic side chain at neutral pH include arginine, histidine and lysine having nitrogen group with pKa high enough to gain proton thereby becoming positively charged in the process. The total basic amino acid (TBAA) of the test and control samples was 10.18 g/100g and 6.02 g/100g respectively. Molasses and urea treatment increased the TBAA by 40.86% of its original level.

The total sulfur amino acid (TSAA) of the samples was 2.40 g/100 and 1.34 g/100g for test and control. Molasses and urea treatment enhanced the TSAA found in the test sample to almost half of the value (5.8 g/100 g) recommended for infants by FAO/WHO [21]. The total aromatic amino acid (ArAA) of 7.49 g/100g found in the test sample was within the stipulated range for ideal infant protein (6.8-11.8 g/100g) set by FAO/WHO while the 5.61 g/100 of the control sample fell short.

TABLE II: DESCRIPTION OF AMINO ACID CONTENT OF TREATED AND UNTREATED GUINEA CORN STALK SILAGE

Amino Acid	Test Sample (g/100g)	Control Sample (g/100g)
TEAA	26.98 (43.32%)	20.48 (46.75%)
TNEAA	35.29 (56.67%)	23.32 (53.24%)
TAAA	13.64 (21.90%)	8.14 (18.58%)
TBAA	10.18 (16.34%)	6.02 (13.74%)
TSAA	2.40 (3.8%)	1.34 (3.05%)
TarAA	7.49 (12.02%)	5.61 (12.80%)

Total Essential Amino Acid (TEAA), Total Amino Acid with Acidic side Chain (TAAA), Total Amino Acid with Basic side Chain (TBAA), Total Sulphur Amino Acid (TSAA), Total Aromatic Amino Acid (TArAA). Treated (test sample), Untreated (control sample).

IV. CONCLUSION

This study showed that molasses and urea treatment improved the silage quality in terms of color, aroma, proximate composition, and amino acid content. Where 14 out of the 18 amino acids determined were significantly elevated at 95% confidence level. The nature of amino acids in the treated silage (test sample) were in the order of prevalence total amino acid with acidic side chain (TAAA) > total amino acid with basic side chain (TBAA) > total aromatic amino acid (TArAA) > total sulfur containing amino acid (TSAA); with isoleucine, valine, alanine, glutamic acid and aspartic acid content meeting up FAO/WHO requirement. Further studies are hereby

recommended to assess the impact of the treated guinea corn stalk on livestock performance.

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CONFLICT OF INTEREST

The authors affirm that they have no particular conflicting monetary interests or personal relationships that could have influenced the work reported in this publication.

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