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Evaluation of Urea and Molasses Treatment on the Nutritional Composition of Gamba Grass (*Andropogon gayanus*) Silage

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ABSTRACT

This study evaluated field grown gamba grass (*Andropogon gayanus*) for its nutritional quality when fermented with molasses, urea and NaCl as additives. The treated (test) and untreated (control) samples were ensiled for 21-day fermentation period. The silages were evaluated for quality characteristics, proximate composition, and amino acids profile, using standard methods. The investigation revealed the mean moisture content as 6.72% and 7.43%, ash content as 9.80% and11.60%, crude protein content as 8.73% and 12.48%, lipid content as 4.52% and 6.89%, fibre content as 15.19% and 16.87% and carbohydrate content as 55.05% and 44.73% for the control and test samples, respectively. There was a significant increase ($p < 0.05$) in ash, protein, lipid, fibre and carbohydrate contents after treatment. The total amino acids values were 58.57 g/100 g protein for the control sample and 67.43 g/100 g protein for the test sample. Glutamic acid content (7.68 and 9.24 g/100 g for the control and test samples, respectively) was the predominant amino acid in gamba grass silage sample, while tryptophan (0.43 and 0.48 g/100 g for the control and test samples, respectively) was the least dominant. Ensiling gamba grass with additives significantly improved the nutritional composition.

Keywords: Gamba grass, silage, treatment, nutritional content

INTRODUCTION

Gamba grass, scientifically known as *Andropogon gayanus* [1], is a leafy grass of tropical Africa origin, mainly used in the production of forage for livestock feeding either when cut or as fresh forage, hay, or silage. However, it is enjoyed by livestock when developing and loses its nutritional value when it matures. Its coarse culms can be used for thatching and mating [2]. The grass is enjoyed by all classes of ruminant livestock. It is palatable when developing and used for continuous and rotational grazing. However, the grass is poor in minerals and low in protein especially when matured [3]

In the tropics, where grazing season fluctuates with availability of pasture grass (gamba grass inclusive) and rainfall, silage making offers a means of improving the utilization of

pastures [4]. However, livestock productivity is currently constrained by complex systemic challenges of which the limited supply of quality feed is commonly cited as the greatest one in most African countries [5]. Limited adoption of silage making is caused by factors including the novelty of forage conservation practices [6], associated costs, poor fit into the prevailing farming systems, and lack of simple and appropriate technologies for ensiling [7]. The primary goal of making silage is to maximize the preservation of original nutrients in the forage crop for feeding of livestock at a later date in livestock feeding programs [8].

Recent increase in livestock production, competition for land resource and herders/farmers clashes where free-range system of animal rearing is practised suggest the need for the development of feed conservation strategies which allow for stock-piling fodders during the time of its abundance for use at the time of scarcity. This will ensure feed supply all year round to meet the nutritional requirements of livestock [9].

Gamba grass, stalks and cereal by-products used as silage materials are mainly energy-valued with less protein value. Hence, they cannot meet the animal nutritional requirements; thereby resulting in loss of weight [10], Silage quality and nutritional value is greatly influenced by biological, ensilage techniques and silage additive used [11, 12]. Silage additives may include natural or industrial products, which are added to forage or grain mass with the aim of controlling the preservation process. This helps to retain as many of the nutrients present in the original fresh forage as possible and to ensure that the growth of lactic bacteria predominates during the fermentation process, since production of lactic acid in quantities helps to ensure good silage [13].

This study is aimed at the evaluation of the effect of urea and molasses additives on the nutritional composition of fermented gamba grass via treatment of the sample with urea and molasses during silage making, determination of proximate composition of treated and the control samples, determination of amino acids profile of treated and control sample and the comparative analysis of the samples (treated and control) using paired sample t-test ($P < 0.05$).

MATERIALS AND METHODS

Sample collection and preparation

The gamba grass samples were collected in a field opposite Ranok Estate, Wukari, Taraba State and were identified in the Department of Crop Production and Protection, Federal University, Wukari, Nigeria. The samples collected were chopped to about 2 – 3 cm in length to ease compaction and consolidation for silage as described by t'Mannetje [14]. The samples were

then separated into two portions (1 kg each) and labelled 'test sample' and 'control sample' respectively. The test sample was allowed to undergo anaerobic fermentation for a period of 21 days, after addition of molasses (1 L), urea (40 g), and salt (5 g) as additives, homogenization, and wrapping in polythene bag. The bag and its content were placed in a pit (15 cm long, 13 cm wide and 60 cm deep) and covered with sand in order to displace air and allow the test sample to ferment in the absence of air [15].

Determination of silage quality

After 21 days, the fermentation was terminated and the silage was opened and air-dried for 1 week before quality assessment in terms of colour, odour, texture, mouldiness, and pH, according to Babayemi and Igbekoyi [16]. Moisture evaporation from the samples was achieved by leaving the samples open in a room condition for two weeks .The samples were later milled and stored in an air-tight container, ready for chemical analysis.

The pH was determined by heating 10 g of the sample in a beaker containing 100 ml of distilled water for 5 min at 60 °C. The liquid was decanted, cooled at room temperature and pH meter (PPD26, UK) was used to determine the level of pH. Colour chat was used to ascertain the silage colour. The odour and smell of the silage was relatively assessed as to whether nice or pleasant or fruity.

Proximate determination

The proximate analyses of the sample for moisture, crude fat, crude fibre, carbohydrate and total ash contents were carried out on the test and control samples using the methods of Association of Official Analytical Chemists [17]. Crude protein was determined by the method developed by Kjeldahl and adopted by the Association of Official Analytical Chemists [18, 19]. The carbohydrate content of the molasses and urea-treated gamba grass was estimated by the difference method. That is, the sum of percentage moisture, ash, crude lipid, crude protein and crude fibre were subtracted from 100% [20, 21]. This procedure was repeated on the control sample.

Amino acid determination

Amino acid content of the test and control samples was determined as described by Association of Official Analytical Chemists with slight modifications [17, 22]. Separation of component amino acid was achieved by means of Ion Exchange Chromatography (IEC) while quantification was done using the Technico Sequential Multi-sample (TSM) Amino Acid Analyzer (Technicon Instruments Corporation, New York). The period of analysis was 76 min

for each sample. The gas flow rate was 0.50 $mLmin^{-1}$ at 60 °C with reproducibility consistent within ± 3%, the net height of each peak produced by the chart recorder of the TSM (each representing an amino acid) was measured and calculated. Amino acid values reported were the averages of two determinations. Norleucine was used as internal standard. Tryptophan was determined after alkali (NaOH) hydrolysis by the colorimetric method. Predicted protein efficiency ratio (P-PER) was determined using the equation described by Adeyeye [23]:

P-PER= -0.468+ 0.454 (Leu) -0.105 (Tyr)

All analysis was done at the Department of Zoology, University of Jos, Nigeria.

Statistical analysis

All analyses were done in triplicate except amino acid content which was done in duplicate. All records and data obtained were analysed 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 ($P < 0.05$).

RESULTS AND DISCUSSION

Physical Characteristics of Samples

The physical attribute of test and control samples in terms of colour, odour, texture and mouldiness was characterized by greenish-yellow colour, fruity odour, firm and dry in texture and not mouldy, while the treated sample was characterized by brownish colour, pleasant odour, firm and dry in texture and not mouldy. Good silage must retain its original colour [24]. The greenish–yellow colour obtained (Plate 1) suggests that the silage was well preserved. The pleasant and fruity odour, firm and dry texture coupled with absence of mould in the silage suggests that the silage was well preserved and lactic acid producing bacteria dominated fermentation process [25, 24].

Fresh gamba grass sample Control sample (Untreated) Test sample (Treated)

Plate 1: Gamba grass untreated and treated samples

The pH value of the samples, as shown in Table 1, was generally acidic: 3.48 and 3.72 for the test and control sample, respectively. The pH values correspond with 3.2 - 3.8 reported on guinea grass and cassava silage [26]. However, the pH value of the test sample is slightly lower an indication of well preserved and good quality silage. The pH value is one of the simplest and quickest ways of evaluating silage quality; the lower the pH, the better the preserved and more stable is the silage. Silage that is properly fermented will have a much lower pH (more acidic to slightly acidic) than the original forage.

Table 1: Silage quality parameters for treated and untreated gamba grass silage

Sample	Colour	Odour	Mouldiness	pH
Test sample	Brown	Pleasant	Not mouldy	3.48
Control sample	Greenish-yellow	Fruity	Not mouldy	3.72
Remark	well preserved	well preserved	well preserved	well preserved

Test sample = Treated gamba grass, Control sample = Untreated gamba grass

Proximate Composition

The proximate composition of the test and control samples, as presented in Table 2, revealed a significant variation in mean ash, fat, fibre, protein and carbohydrate contents between the test and control sample of gamba grass silage due to molasses and urea treatment ($p < 0.05$).

The mean moisture content of the test sample was 7.43% while the control was 6.72% indicating no significant difference ($p > 0.05$) due to molasses and urea treatment. That only slightly higher moisture content was found in the test sample indicates lower stability to microbial action and spoilage. The mean moisture content of the test sample was in agreement with the 6.72 – 7.43% obtained in ensiled maize stovers [27].

The mean ash contents of the test and control gamba grass silages were 11.50% and 9.80% respectively, with significant elevation ($p < 0.05$) in ash content due to molasses and urea treatment. The 11.50% ash content of the test sample gamba grass silage was in agreement with the 11.50% ash content found in Alfalfa hay and two-fold the 5.48% content of corn silage [28].

The mean crude lipid contents of the test and control gamba grass silage were 6.89% and 4.52% respectively, with a significant elevation ($p < 0.05$) in crude lipid content due to urea and molasses treatment. The crude lipid contents were all higher than the 2.4% content found in corn crop silage [29].

Crude fibre is generally defined as the macromolecules present in the diet that resist digestion by human endogenous enzymes and is essentially composed of plant cell wall remnants, such as cellulose, hemicelluloses, pectic polysaccharides and lignin [30]. Rather, they aid to speed up the rate of digestion in animals. The mean crude fibre contents of the test and control samples were respectively 16.87% and 15.19%, revealing a significant elevation ($p < 0.05$) in crude fibre content due to molasses and urea treatment at 95% confidence limit. Singh and Oosting [31] classified roughage feeds with crude fibre content less than 45% as high quality, those with values within 45 - 65% as medium quality while those with fibre content higher than 65% as low quality. This implies that both the test and control gamba grass silage are of high quality.

The mean crude protein content in the test and control gamba grass samples were 12.48% and 8.73% respectively, revealing a significant increase in crude protein content due molasses and urea treatment at 95% confidence limit ($P < 0.05$). The 8.73% crude protein content of the control sample correspond with the 7 - 10% content of fresh gamba grass grown on moderately fertile soils and higher than the 2% content when mature, while the 12.48% content of the test and treated gamba grass sample was much higher [3].

The mean carbohydrate contents of the test and control gamba grass silage sample were 44.73% and 55.05% respectively, revealing a significant decrease in carbohydrate content due urea and molasses treatment at 95% confidence limit ($P < 0.05$). The carbohydrate content of the test and control gamba grass silage were all less than the 69.89% found in pulp of desert date [22].

Table 2: Mean proximate composition of gamba grass silage

Parameters (%)	Test sample	Control sample	Sig. Difference (P)
Moisture	7.43±0.15	6.72±0.54	0.18
Ash	11.60±0.36	9.80±0.31	0.01
Protein	12.48±0.14	8.73±0.36	0.00
Lipid	6.89±0.09	4.52±0.62	0.03
Fibre	16.87±0.32	15.19±0.16	0.02
Carbohydrate	44.73±0.81	55.05±1.28	0.00

P = probability value ($p < 0.05$); Test sample = Treated gamba grass, Control sample = Untreated gamba grass

Amino Acids Composition

Table 3 reveals that all the 18 amino acid determine were present in gamba grass samples, both in the test sample (treated) and in the control sample (untreated). The mean content of amino acids control samples was in order of abundance: Glutamic acid > Leucine > Aspartic acid > Phenylalanine = Glycine > Lysine > Arginine > Alanine > Valine > Proline > Serine > Threonine > Isoleucine > Tyrosine > Histidine > Methionine > Cystine > Tryptophan. However, there was a slight shift in order of abundance due to molasses and urea treated as reflected in the test sample: Glutamic acid > Leucine > Aspartic acid > Arginine > Alanine > Phenylalanine > Glycine > Lysine > Valine > Threonine = Proline > Serine > Isoleucine > Tyrosine > Histidine > Cystine > Methionine > Tryptophan. Glutamic acid, the most prevalent in both the test and control sample tryptophan was found to be the least.

The predicted protein efficiency ratio (P–PER) is one of the quality parameters used for protein evaluation [32, 22], experimentally within the scale of 0.0 for a very poor protein to a maximum possible of 4.0 [33, 34]. The 1.88 predicted protein efficiency ratio found in the test sample was higher than the 1.74 recorded in the control sample. Both values were higher than the 1.67 ratio found in dung beetle silage [35].

Table 3: Mean amino acids content and paired t-test inferential between the untreated and treated gamba grass

Amino acid	Control sample (g/100 g)	Test sample (g/100 g)	Paired t-test
Leucine	5.43	5.83	0.00
Lysine	4.00	4.10	0.34
Isoleucine	2.89	3.38	0.00
Phenylalanine	4.04	4.18	0.12
Tryptophan	0.43	0.48	0.06
Valine	3.20	3.69	0.01
Methionine	1.09	1.16	0.00
Proline	3.03	3.52	0.05
Arginine	3.42	4.20	0.03
Tyrosine	2.37	2.83	0.16
Histidine	0.00	2.37	0.02
Cystine	0.34	2.06	0.04
Alanine	0.00	4.19	0.02
Glutamic acid	0.12	9.24	0.00
Glycine	0.06	4.15	0.10
Threonine	2.93	3.52	0.00
Serine	3.01	3.50	0.01
Aspartic acid	4.86	5.03	0.04
Total	58.57	67.43	1.00
P-PER	1.74	1.88	

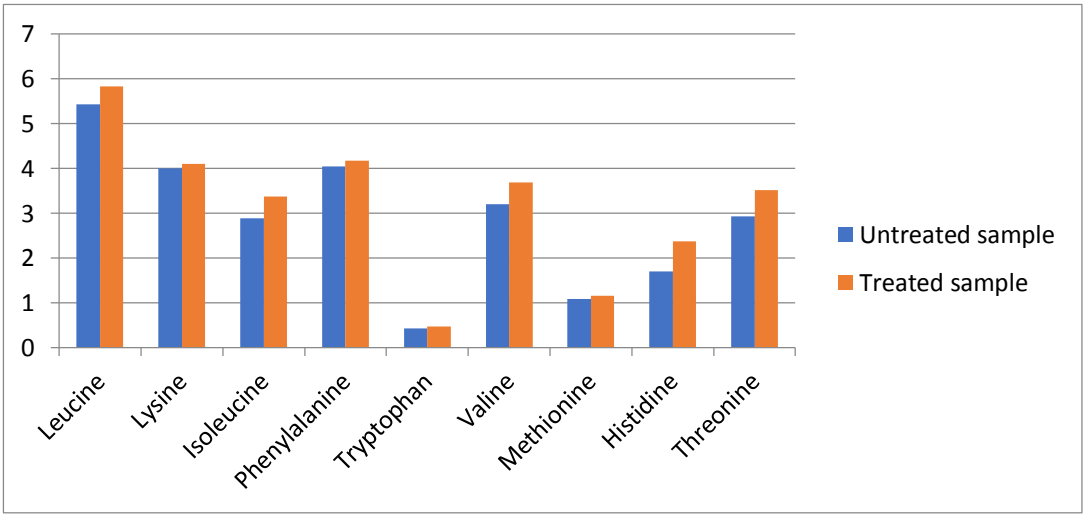
P value < 0.05 = Significant difference, Test sample = Treated gamba grass, Control sample = Untreated gamba grass, P-PER = Predicted protein efficiency ratio

Essential amino acids content

This includes the group of amino acids which the body of animals cannot synthesize. They are often obtained from food consumed by animals. They are: leucine, lysine, isoleucine, valine, methionine, phenylalanine, histidine, tryptophan and threonine. The most abundant essential amino acids were leucine followed by phenylalanine and lysine in both the control and test sample concentrations, 5.43, 4.04 and 4.00 g/100 g protein for the control sample and 5.83, 4.18 and 4.10 g/100 g protein in the test sample respectively. Treatment by means of molasses and urea increased the essential amino acid content as demonstrated in Figure 1. This agrees with the report that cooking and roasting results in a slight increase in leucine and lysine concentration of black turtle bean [36]. This was in contrast to the report of Amaechi and Oluagha [37], recording a small decrease in lysine in *Artocarpus heterophyllus* seeds when treated by boiling.

Leucine the most abundant essential amino acid in gamba grass is highly functional and it activates the mammalian target of rapamycin pathway to regulate protein synthesis and catabolism in the body [38, 39]. It has been reported to promote the development of the gastrointestinal tract in animals [40].

Previous studies by researchers have shown that leucine and phenylalanine can regulate pancreatic enzyme synthesis and promote starch digestion in adult ruminants [41]. Persistently low leucine levels can result in decreased appetite, poor feeding, lethargy, poor growth, weight loss, skin rashes, hair loss, and desquamation, while prolonged phenylalanine deficiency causes growth failure and loss of muscle mass in animals.



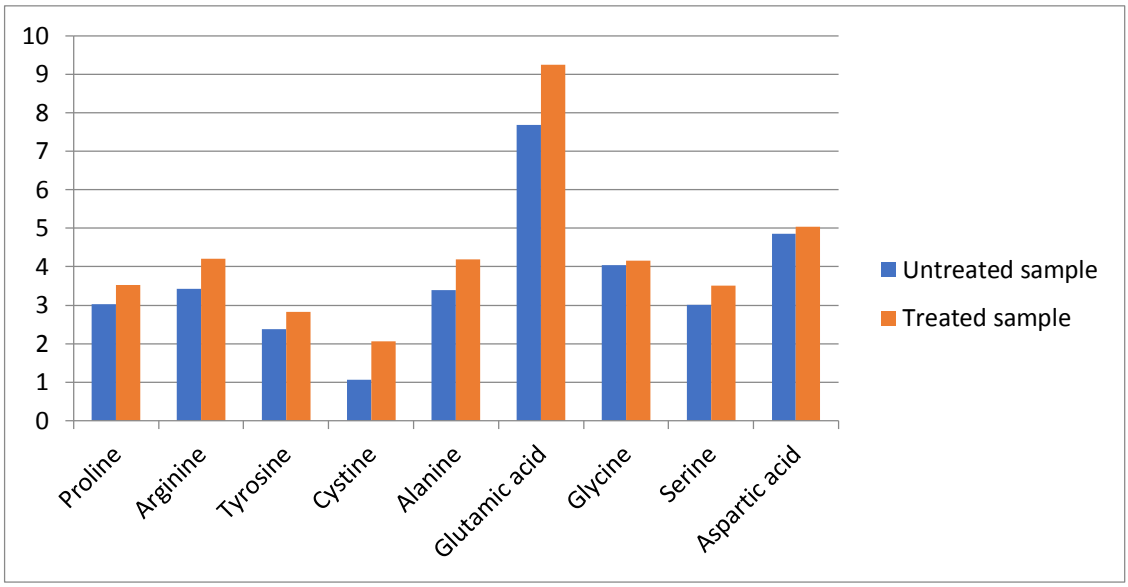
Test sample = Treated gamba grass, Control sample = Untreated gamba grass

Figure 1: Effect of processing on essential amino acid content of gamba grass silage

Non-essential amino acids contents

This group of amino acids are synthesized by the body. Hence, there may be no need of obtaining them from external source under normal conditions. They include: proline, cysteine, arginine, aspartic acid, glutamic acid, alanine, serine, tyrosine and glycine.

The most abundant non-essential amino acid was glutamic acid in both the test and control samples. Glutamic acids as well as other non-essential amino acids play an important role during protein synthesis, metabolism and digestion, regulation of gene expression, in anti-oxidative responses, and immune responses.



Test sample = Treated gamba grass, Control sample = Untreated gamba grass

Figure 2: Effect of processing on non-essential amino acid content of gamba grass silage

Description of amino acids in gamba grass 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 sulphur containing amino acid (TSAA) as presented in Table 4.

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 14.27 g/100g and 12.54 g/100g crude protein 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 [35]. However, molasses and urea treatment increased the TAAA by 13.79% 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) values of the test and control samples were10.67 g/100g and 9.12 g/100g respectively. Molasses and urea treatment increased the TBAA by 40.86% of its original level.

The total sulphur amino acid (TSAA) values of the samples were 3.22 g/100 and 2.15 g/100g for test and control respectively. Molasses and urea treatment enhanced the TSAA found in the test sample to more than half of the value (5.8 g/100 g) recommended for infants by FAO/WHO [34]. The total aromatic amino acid (TArAA) of 7.49 g/100g found in the test sample as well as the 6.84 g/100g in the control sample both fall within the stipulated range for ideal infant protein (6.8-11.8 g/100g) set by FAO/WHO. The original TSAA content was increase by 9.50% due to treatment. Phenylalanine the major component of total aromatic amino acids in gamba grass promotes the secretion of cholecystokinin (CCK) through calcium-sensing receptors [42]. CCK stimulates the pancreas to synthesize pancreatic amylase, trypsin and trypsinogen. CCK also stimulates the release of pancreatic enzymes and enhanced pancreatic enzyme activity [43].

Table 4: Description of amino acid content of treated and untreated gamba grass silage

Amino Acid	Test Sample (g/100g)	Control Sample (g/100g)
TEAA	28.71	25.71
TNEAA	38.72	32.86
TAAA	14.27	12.54
TBAA	10.67	9.12
TSAA	3.22	2.15
TArAA	7.49	6.84

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)

CONCLUSION

The use of additives (urea, molasses and NaCl) for silage making has beneficial effect on silage quality and nutritional composition of gamba grass (*A. gayanus*). This study shows that there is significant difference ($P < 0.05$) in most of the values obtained from the analysis carried out between the control and the treated samples. The prevalence of amino acids pattern of gamba grass was in the order: TAAA > TBAA > TArAA > TSAA, with non-essential amino acid dominating the essential amino acid content. Therefore, silage making with the inclusion of urea, molasses and NaCl is important and can be used to supply farm animals with the moderate nutrition they require in the time of deficiency.

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