Nig. J. Biotech. Vol. 40(1): 29-42 (June 2023)
ISSN: 0189 1731
Available online at
http://www.ajol.info/index.php/njb/index
and https://bsn.org.ng
DOI: https://dx.doi.org/10.4314/njb.v40i1.4

NIGERIAN JOURNAL OF BIOTECHNOLOGY

Biochemical Assessment of Solid-State Fermented Elephant Grass and its Potential Incorporation in Broiler's Diets

E.P. Eghagha[a], E.O. Egbune[a,c*], T. Ezedom[b], O.C Orororo[b], A.A. Anigboro[a], N.J. Tonukari[a,c]

[a]Department of Biochemistry, Faculty of Science, Delta State University, P.M.B. 1, Abraka, Nigeria.
[b]Department of Medical Biochemistry, Faculty of Basic Medical Sciences, Delta State University, P.M.B. 1, Abraka, Nigeria.
[c]Tonukari Biotechnology Laboratory, Sapele Delta State, Nigeria

Abstract

The objective of this study was to determine if incorporating pre-treated ensiled elephant grass into broiler feed could help meet the rising demand for animal protein. The elephant grass underwent solid-state fermentation for seven days and ensiling in airtight nylon bags for 21 days. Compared to the control, pre-treated ensiled elephant grass exhibited a significant increase in soluble protein concentration ($p<0.05$) during fermentation. However, the glucose concentration was significantly lower ($p>0.05$) in the pre-treated ensiled elephant grass after fermentation than in the control. Elephant grass that was solid-state fermented and ensiled with *Rhizopus oligosporus* demonstrated the highest percentage inhibition (22.4±0.5%). The pre-treated ensiled elephant grass had significantly higher mannanase activity ($p<0.05$) than the control. In terms of serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities, there were no significant differences between the control group and the experimental group fed with diets composed of ensiled elephant grass, ensiled elephant grass fermented with *R. oligosporus*, or solid-state fermented and ensiled elephant grass with *R. oligosporus* and urea in a 25% maize replacement. In conclusion, ensiled elephant grass can replace 25% maize in broiler starter feed, and the animal feed industry could benefit from the mannanase identified in this study.

Keywords: Elephant grass, Fermentation, Rhizopus oligosporus, Soluble protein, Animal feed
***Corresponding authors email:** egbuneegoamaka@gmail.com

Introduction

The world's increasing population demands the utilization of existing livestock resources to meet the demand for animal protein, but this is only possible if optimal forage and fodder production is ensured (Weishaupt et al., 2020; Ezedom et al., 2022). Availability of quality feed and forage resources are essential for sustainable livestock production (Negawo et al., 2017; Naah et al., 2019; Bakare et al., 2020). One of the major challenges faced by livestock producers in tropical countries is providing proper nutrition for their animals, especially during the dry season when pasture and cereal residues are limited in quantity and nutritional quality (Tonukari et al., 2016; Michalk et al., 2018; Kebede 2020).

Pennisetum purpureum Schumach, commonly known as elephant grass, is a monocot species belonging to the Poaceae family and Pennisetum genus. It is widely used as forage in tropical and subtropical regions and is a major source of livestock feed for cattle, dairy, and sheep in West Africa (Bakare et al., 2020). Due to the small and fragmented landholdings of most smallholder livestock producers, elephant grass

is a preferred alternative to other feed options as it is a high-yielding forage that requires minimal inputs and acreage (Negawo et al., 2017; Ojo et al., 2022; Ndego et al., 2023).

There is potential for enhancing the nutritional quality of elephant grass through the application of advancements observed in other fodder crops (Simeão et al., 2021; Chandel et al., 2021). Improving the digestibility and nutritional value of elephant grass could significantly boost livestock productivity. However, the existing challenges in terms of palatability, nutritional quality, propagation via seed or vegetative organs, and diseases like elephant grass stunt and head smut significantly affect its production in some regions of Africa (Makini et al., 2019; Mutwedu et al., 2020; Egbune et al., 2022).

Improving the utilization of elephant grass requires the use of appropriate processing methods to enhance its nutritive value. Solid-state fermentation (SSF) is one such method used to improve the bioavailability of nutrients and increase the nutritive value of animal feed (Olukomaiya et al., 2020; Egbune et al., 2021a). The aim of this study is to enhance the nutritional value of elephant grass for use as animal feed and for the production of mannanase. The study examines the biochemical evaluation of pre-treated ensiled elephant grass and its potential use in broiler diet.

Materials and method

Collection of plant material

Elephant grass (Pennisetum purpureum Schumach) samples were collected and verified by Mr. Micheal E.O. of the Botany Department at Delta State University, Abraka. The collected samples were ground and stored at room temperature. *R. oligosporus* strains were obtained from Tonukari Biotechnology laboratory sited at Songhai in Amukpe, Sapele, Delta State. A voucher number (DELSU 112) was provided for the Elephant grass sample.

Various pretreatment for elephant grass

To enhance the nutritional quality of elephant grass, various pretreatments were applied, including ensiling and solid-state fermentation with the addition of *R. oligosporus* and/or urea. The ensiling process involves storing the elephant grass with 10% water in air-tight nylon bags for 21 days, followed by 7 days of solid-state fermentation. The solid-state fermentation process involves adding 10% water and/or 10% *R. oligosporus* and/or 1.2% urea to the elephant grass and then subjecting it to fermentation for 3 days, ensiling for 18 days, and then another 7 days of solid-state fermentation. After each process, the fermented mixture is dried and ground into small pieces. The following are various pretreatments for elephant grass, along with their respective acronyms:

E: Elephant grass: Unfermented elephant grass: This does not involve any processing steps, and the elephant grass is fed as it is, without any fermentation or additives.

fE: Ensiled elephant grass - In this pretreatment, 10% water is added to the elephant grass, and the mixture is ensiled for 21 days in air-tight nylon bags, followed by 7 days of solid-state fermentation. After that, the fermented mixture is dried and ground into small pieces.

fEH: Ensiled elephant grass with *R. oligosporus* - Elephant grass + 10% *R. oligosporus* + 10% water: This process involves adding 10% water and 10% *R. oligosporus* to the elephant grass. The mixture is ensiled for 21 days in air-tight nylon bags, followed by 7 days of solid-state fermentation. After that, the fermented mixture is dried and ground into small pieces.

fEU: Ensiled elephant grass with urea - Elephant grass + 1.2% urea in 10% water: This process involves adding 1.2% urea to 10% water, and then adding the mixture to the elephant grass. The mixture is ensiled for 21 days in air-tight nylon bags, followed by 7 days of solid-state fermentation. After that, the fermented mixture is dried and ground into small pieces.

fEHU: Ensiled elephant grass with *R. oligosporus* and urea - Elephant grass + 1.2% urea in 10% water: This process involves adding 1.2% urea to 10% water, and then adding the mixture to the elephant grass. The mixture is ensiled for 21 days in air-tight nylon bags, followed by 7 days of solid-state fermentation. After that, the fermented mixture is dried and ground into small pieces.

fsEH: Solid state fermented and ensiled elephant grass with *R. oligosporus* - Elephant grass +

10% *R. oligosporus* + 10% water: In this process 10% water and 10% *R. oligosporus* are added to the elephant grass. The mixture is then subjected to solid-state fermentation for 3 days, ensiled for 18 days, and then subjected to another 7 days of solid-state fermentation.
fsEHU: Solid state fermented and ensiled elephant grass with *R. oligosporus* and urea - Elephant grass + 10% HPY + 1.2% urea in 10% water: This process involves adding 10% *R. oligosporus*, 1.2% urea, and 10% water to the elephant grass. The mixture is then subjected to solid-state fermentation for 3 days, ensiled for 18 days, and then subjected to another 7 days of solid-state fermentation.

Biochemical parameters
Total soluble protein was determined using the procedure described by Gornall et al. (1949) with bovine serum albumin as the standard. The glucose content was estimated using the Randox glucose kit and following the manufacturer's instructions. Reducing sugars were estimated using the 3,5-dinitrosalicylic acid (DNS) colorimetric technique (Miller 1959). The total phenol content was determined by the procedure described by Singleton and Rossi (1965), using catechin as the standard. The total flavonoid contents were determined by colorimetry following the assay procedure described by Jia et al. (1999). The study utilized the DPPH assay, as described by Hatano et al. (1988), to determine the antioxidant activity of elephant grass. The reducing power of the extracts was examined using the procedure described by Benzie and Strain (1996). The modified methods of Araujo and Ward (1990) were used to determine the mannanase activity.

Experimental design, feed formulations and trial
In this study, eight different broiler starter feed diets were formulated by substituting 25% of maize with pretreated elephant grass while keeping all other ingredients constant (see Table 1. for detailed diet compositions). The study involved a total of forty newly hatched Cornish White x White Plymouth Rock broiler chicks, which were divided into eight groups of eight chicks each and fed the respective diets for a duration of four weeks. Throughout the trial, the chicks had free access to feed and water. To determine their weight gain, the chicks' weights were recorded on the first day of the experiment and at the end of the fourth week, and the difference in weight (g) between the two measurements was calculated. Venous blood was collected from the chicks' wings and/or legs using sterile syringes and needles, and serum was obtained by centrifuging the clotted blood at 2000 g for 10 minutes. The serum was stored at 4°C for subsequent biochemical analyses.

Table 1. Replacement of 25% maize with fermented elephant grass (ensiled and SSF)

Starter	**Control**	**E**	**fE**	**fEH**	**fEU**	**fEHU**	**fsEH**	**fsEHU**
Maize	5.2	3.9	3.9	3.9	3.9	3.9	3.9	3.9
Elephant Grass	0	1.3	1.3	1.3	1.3	1.3	1.3	1.3
Palm kernel cake	0.8	0.8	0.8	0.8	0.8	0.8	0.8	0.8
Soybean cake	3.5	3.5	3.5	3.5	3.5	3.5	3.5	3.5
Bone meal	0.148	0.148	0.148	0.148	0.148	0.148	0.148	0.148
Limestone	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Dicalcium phosphate	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Lysine	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025
Methionine	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
Premix	0.025	0.025	0.025	0.025	0.025	0.025	0.025	0.025
Enzymes	0.002	0.002	0.002	0.002	0.002	0.002	0.002	0.002
Salt	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Total (kg)	10	10	10	10	10	10	10	10

E-Elephant grass, fE-Ensiled Elephant grass, fEH-Ensiled Elephant grass with *R. oligosporus*, fEU-Ensiled Elephant grass with urea, fEHU-Ensiled Elephant grass with *R. oligosporus*, fsEH-Solid state fermented

and ensiled elephant grass with *R. oligosporus*, fsEHU-Solid state fermented and ensiled elephant grass with *R. oligosporus*.

Weight gain measurement

Each chick's weight was measured at the start of the experiment and after four weeks to calculate their weight gain. In an effort to develop a cost-effective, nutritious, and healthy feed, 25% of the maize content in the broiler starter feed was substituted with pretreated elephant grass.

Evaluation of the biochemical effect (s) of feed formulations

The serum levels of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) were determined as biochemical indices. The assays for these indices were conducted following the instructions provided in their respective RANDOX assay kits (Randox Laboratories Ltd, UK).

Statistical analysis

Data obtained were subjected to statistical analysis using one-way ANOVA (analysis of variance) and Fischer's test of least significance (LSD); values are presented as Mean ± Standard deviation. A significance level of 0.05 ($p<0.05$) was used to determine the statistical significance of the results with a 95% confidence level.

Results

The pre-treated ensiled elephant grass showed a significant ($p<0.05$) increase in soluble protein concentration following fermentation compared to the control, as depicted in Figure 1. The solid-state fermented and ensiled elephant grass using *R. oligosporus* exhibited the highest concentration of soluble protein, which was measured at 26.4±0.6mg/g. Figure 1 presents the glucose concentration of pre-treated ensiled elephant grass, which decreased significantly ($p<0.05$) due to fermentation when compared to the control. Similarly, Figure 1 shows a significant decrease ($p<0.05$) in the reducing sugar concentration of pre-treated ensiled elephant grass compared to the control.

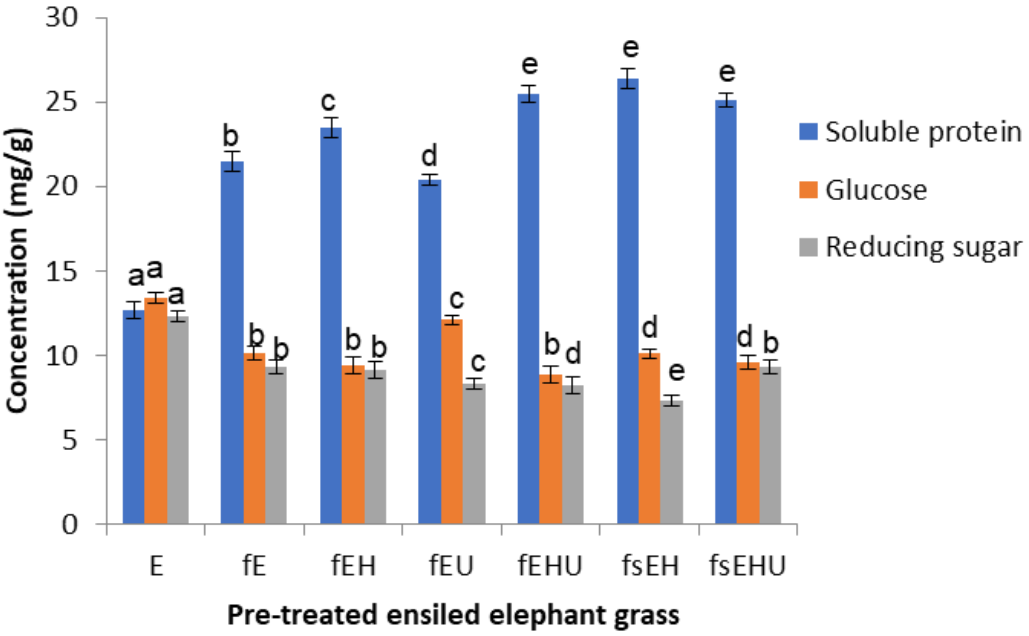

Fig 1: Soluble protein, glucose and reducing sugar concentration of pre-treated ensiled elephant grass. Different treatments were applied to elephant grass, including E-Elephant grass, fE-Ensiled Elephant grass, fEH-Ensiled Elephant grass with *R. oligosporus*, fEU-Ensiled Elephant grass with urea, fEHU-Ensiled Elephant grass with *R. oligosporus*, fsEH-Solid state fermented and ensiled elephant grass with *R. oligosporus*, and fsEHU-Solid state fermented and ensiled elephant grass with *R. oligosporus* and Urea. The results indicated that values with the letter "a" did not show significant differences ($p > 0.05$) from the control, whereas values with the letters "b", "c", "d", and "e" demonstrated significant differences ($p < 0.05$).

Figure 2 shows the free radical scavenging inhibition activities of pre-treated ensiled elephant grass, which exhibited a significant increase ($p<0.05$) compared to the control. The FRAP percentage inhibition of pre-treated ensiled elephant grass also showed a significant increase ($p<0.05$) compared to the control. The solid state fermented and ensiled elephant grass with *R. oligosporus* showed the highest percentage inhibition of 22.4±0.5 (%).

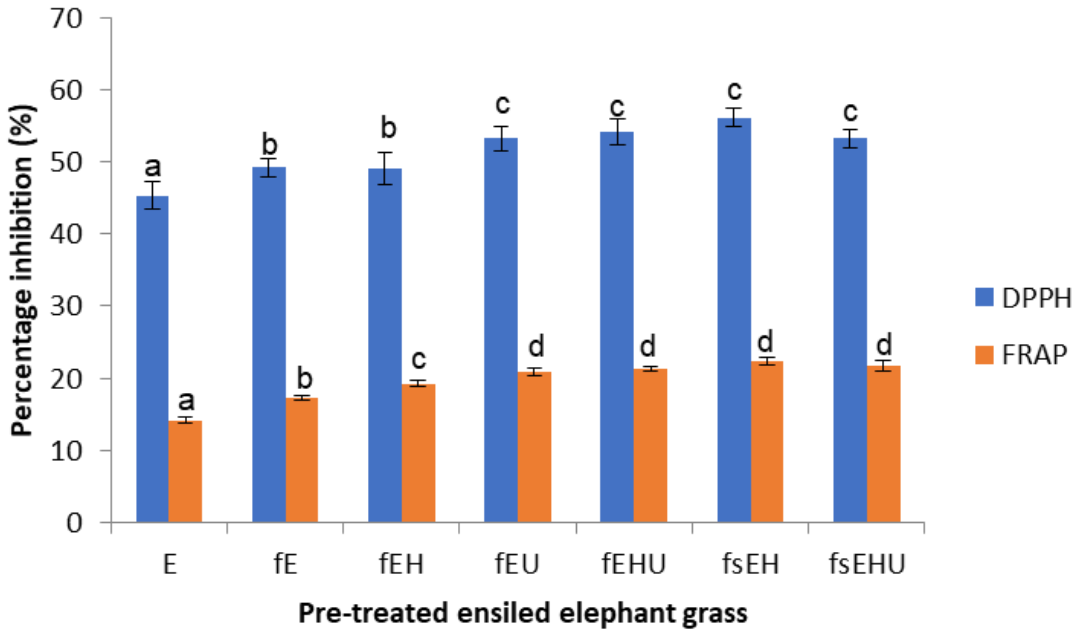

Fig 2: Free radical scavenging inhibition and FRAP percentage inhibition of pre-treated ensiled elephant grass. Different treatments were applied to elephant grass, including E-Elephant grass, fE-Ensiled Elephant grass, fEH-Ensiled Elephant grass with *R. oligosporus*, fEU-Ensiled Elephant grass with urea, fEHU-Ensiled Elephant grass with *R. oligosporus*, fsEH-Solid state fermented and ensiled elephant grass with *R. oligosporus*, and fsEHU-Solid state fermented and ensiled elephant grass with *R. oligosporus* and Urea. The results indicated that values with the letter "a" did not show significant differences ($p > 0.05$) from the control, whereas values with the letters "b", "c", "d", and "e" demonstrated significant differences ($p < 0.05$).

In this study, various pre-treatments were examined to determine their effect on the total phenolic and flavonoid content of ensiled elephant grass. As indicated in Figure 3, the solid state fermentation and ensiling of elephant grass with *R. oligosporus* and urea resulted in the highest total phenolic content (32.8±0.6 µg/ml), which was significantly different ($p<0.05$) from the control (25.1±0.5 µg/ml) and other pre-treatments. All pre-treatments showed a significant increase ($p<0.05$) in total phenolic content compared to the control. Figure 3 also displays the findings of the total flavonoid content determination of pre-treated ensiled elephant grass. There was no significant difference ($p>0.05$) in the flavonoid content of pre-treated ensiled elephant grass compared with the control. However, solid state fermented and ensiled elephant grass with *R. oligosporus* and solid state fermented and ensiled elephant grass with *R. oligosporus* and urea differed significantly ($p<0.05$) in their flavonoid content.

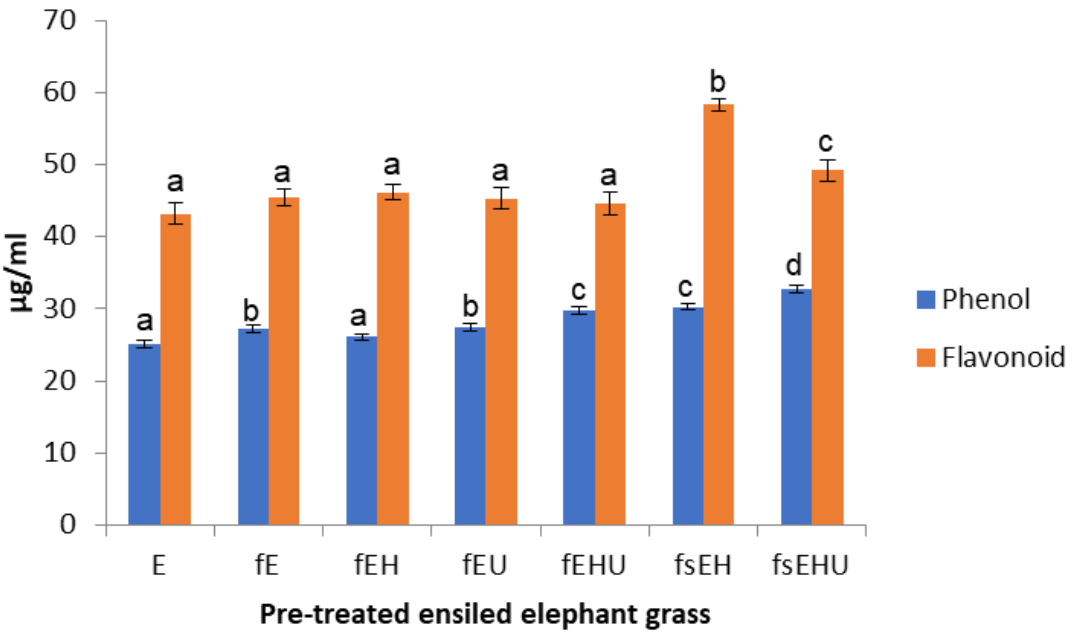

Fig 3: Total phenol and Total flavonoid content of pre-treated ensiled elephant grass. Different treatments were applied to elephant grass, including E-Elephant grass, fE-Ensiled Elephant grass, fEH-Ensiled Elephant grass with *R. oligosporus*, fEU-Ensiled Elephant grass with urea, fEHU-Ensiled Elephant grass with *R. oligosporus*, fsEH-Solid state fermented and ensiled elephant grass with *R. oligosporus*, and fsEHU-Solid state fermented and ensiled elephant grass with *R. oligosporus* and Urea. The results indicated that values with the letter "a" did not show significant differences ($p > 0.05$) from the control, whereas values with the letters "b", "c", "d", and "e" demonstrated significant differences ($p < 0.05$).

Figure 4 displays the mannanase activities of the pre-treated ensiled elephant grass compared to the control and ensiled elephant grass. The results indicate a significant increase ($p<0.05$) in the mannanase activity of the pre-treated ensiled elephant grass when compared to the control. However, there was no significant difference between the mannanase activity of the ensiled elephant grass and the control.

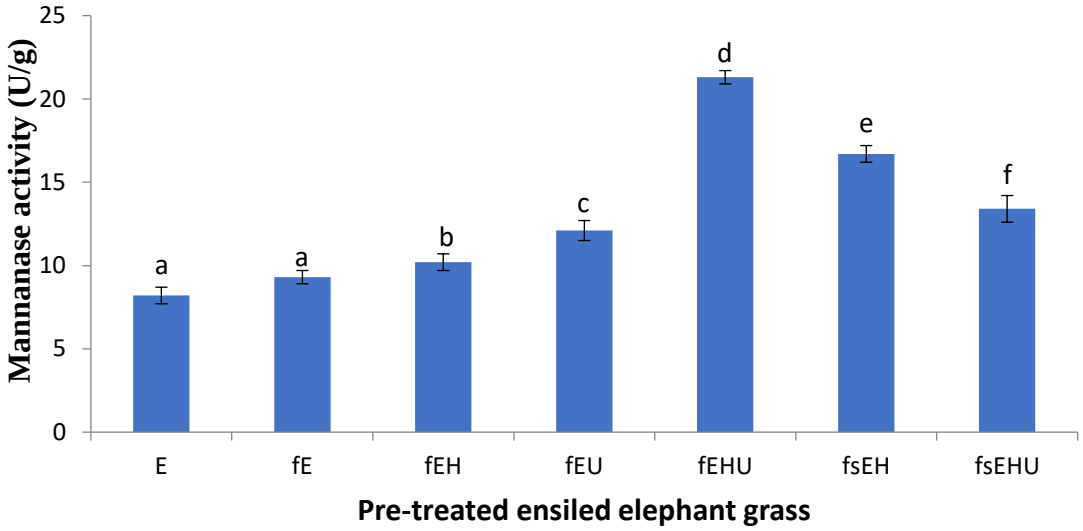

Fig 4: Mannanase activity of pre-treated ensiled elephant grass. Different treatments were applied to elephant grass, including E-Elephant grass, fE-Ensiled Elephant grass, fEH-Ensiled Elephant grass with *R. oligosporus*, fEU-Ensiled Elephant grass with urea, fEHU-Ensiled Elephant grass with *R. oligosporus*, fsEH-Solid state fermented and ensiled elephant grass with *R. oligosporus*, and fsEHU-Solid state fermented and ensiled elephant grass with *R. oligosporus* and Urea. The results indicated that values with the letter "a" did not show significant differences ($p > 0.05$) from the control, whereas values with the letters "b", "c", "d", and "e" demonstrated significant differences ($p < 0.05$).

The results presented in Figure 5 show that there was no significant difference in weight gain observed in the birds that were maintained on ensiled elephant grass, ensiled elephant grass with *R. oligosporus* and solid state fermented and ensiled elephant grass with *R. oligosporus* and urea. However, birds that were maintained on all other diets exhibited a significant reduction.

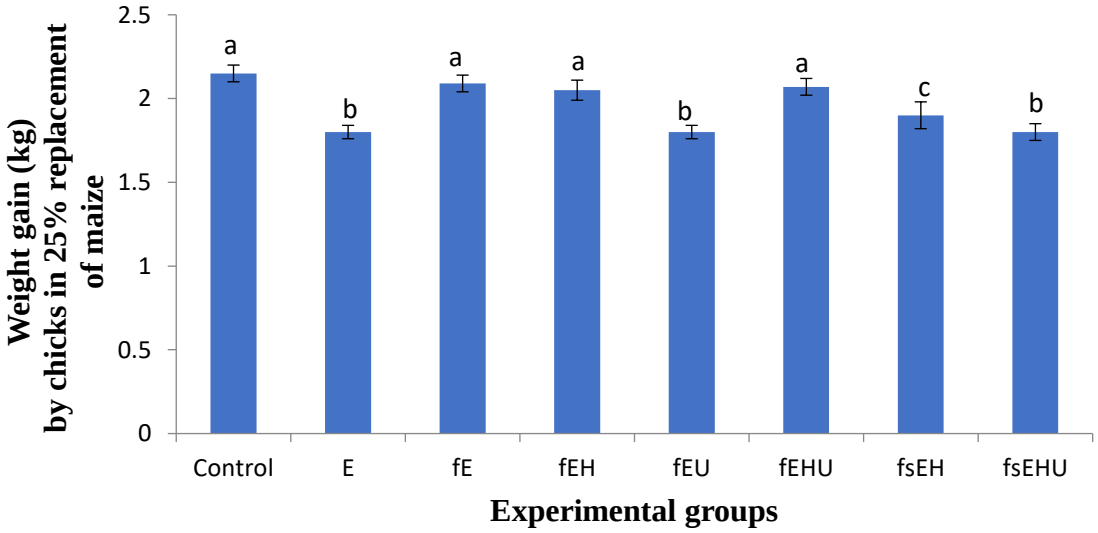

Fig 5: Weight gained (kg) by the chicks in 25% replacement of maize.Each bar in the graph represents the mean value ± standard deviation of five replications (n=5). Different letters used to label the bars indicate significant differences at $p<0.05$. The different treatments are represented as follows: E (Elephant grass), fE (Ensiled Elephant grass), fEH (Ensiled Elephant grass with *R. oligosporus*), fEU (Ensiled Elephant grass with urea), fEHU (Ensiled Elephant grass with *R. oligosporus*), fsEH (Solid state fermented and ensiled elephant grass with *R. oligosporus*), and fsEHU (Solid state fermented and ensiled elephant grass with *R. oligosporus* and Urea).

Figure 6 presents the serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities (measured in U/L) of the birds. The findings revealed no significant difference in the activities of these enzymes between the control diet and the experimental diets. However, there was a significant increase ($p<0.05$) in the alanine aminotransferase (ALT) levels of birds fed ensiled elephant grass with urea compared to the control diet.

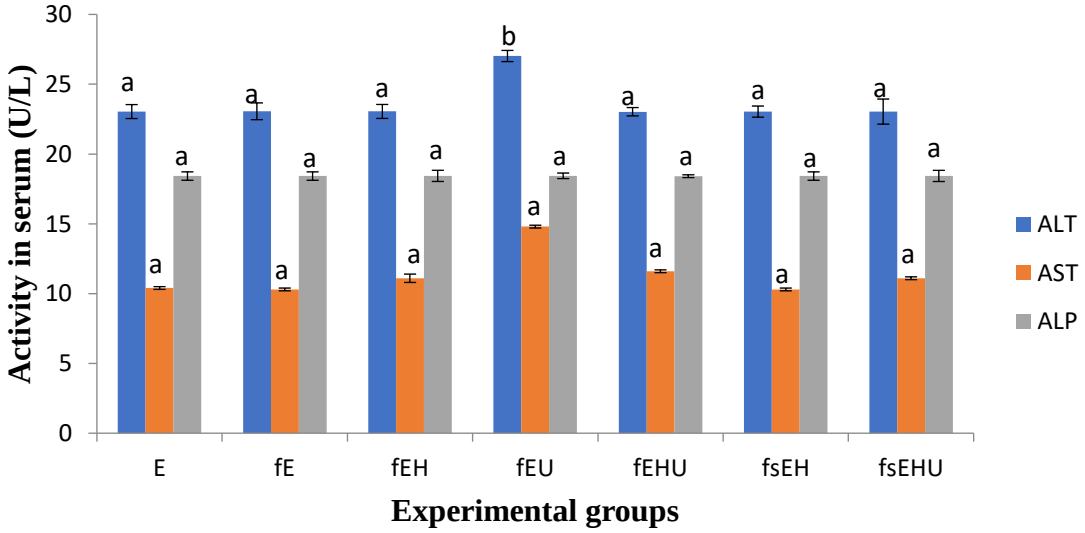

Fig 6: AST, ALT and ALP activity in the serum of broiler chicks fed with formulated diet Each bar is an expression of mean ± SD, an equivalent of five replications (n = 8), and designation of each bar with different letters showed marked significant difference at $p < 0.05$. Each bar in the graph represents the mean value ± standard deviation of five replications (n=5). Different letters used to label the bars indicate significant differences at $p<0.05$. The different treatments are represented as follows: E (Elephant grass), fE (Ensiled Elephant grass), fEH (Ensiled Elephant grass with *R. oligosporus*), fEU (Ensiled Elephant grass with urea), fEHU (Ensiled Elephant grass with *R. oligosporus*), fsEH (Solid state fermented and ensiled elephant grass with *R. oligosporus*), and fsEHU (Solid state fermented and ensiled elephant grass with *R. oligosporus* and Urea).

Discussion

During the solid-state fermentation and ensiling of elephant grass with *R. oligosporus*, there was a significant increase in the concentration of soluble proteins compared to the control. This process of solid-state fermentation has numerous benefits, including increased amino acid and soluble protein content, decreased mycotoxins, and prevention of oxidative rancidity. Additionally, changes in the concentrations of protein, fat, and carbohydrates indicate that the enzymatic activity of microorganisms alters the texture, flavor, and nutritional composition of the fermented product (Londoño-Hernandez et al., 2018). Similar results have been observed in

previous studies that investigated the solid-state fermentation of maize offal with *R. oligosporus*. The increased protein content in the fermented maize cob is believed to be due to the secretion of extracellular enzymes, such as amylases, linamarase, and cellulase, by the fermenting organisms as they utilize cassava starch as a carbon source. These findings are consistent with the results of Anigboro et al. (2020).

In this study, the concentration of glucose in pre-treated ensiled elephant grass was significantly lower after fermentation than in the control group ($p>0.05$). This is in contrast to a study by Anigboro et al. (2020), which found an increase in glucose content in maize cob after fermentation with *R. oligosporus*. The findings of Anigboro et al. (2020) indicate that solid-state fermentation can increase glucose concentration at different inoculum sizes. During the fermentation process, the fungus absorbs simple sugars and starts breaking down starch chains into multiple pieces through the action of enzymes such as amylase, which increases the sugar level. This hydrolysis of starch leads to the release of glucose, which is the main reducing sugar and causes an increase in this type of sugar for all tested treatments, while the fungus is growing in the substrate. As a result, microorganisms produce specific extracellular enzymes that break down complex polysaccharides to glucose throughout the fermentation process, leading to an increase in glucose concentration.

The concentration of reducing sugar in pre-treated ensiled elephant grass decreased significantly ($p>0.05$) compared to the control. The breakdown of starch during fermentation caused the reducing sugar in the fermented product to decrease. Previous studies have shown that higher levels of reducing sugars increase the susceptibility of starch to hydrolysis, which suggests that consuming fermented agricultural byproducts may be beneficial for people with diabetes (Budhwar et al., 2020; Baniwal, et al., 2021; Qin et al., 2022).

The study found that pre-treated ensiled elephant grass had significantly higher DPPH free radical-scavenging ability than the control. These results align with earlier research that has shown solid-state fermentation (SSF) to be a promising technology for enhancing the antioxidant potential of fermented agricultural byproducts, such as cassava stems, due to their rich polyphenolic compounds that can neutralize reactive oxygen species (ROS) generated during the lipid peroxidation process (Jogawat et al., 2021; Sharma et al., 2021). The polyphenolic compounds in the fermented elephant grass may act as proton donors to ROS, terminating the lipid peroxidation chain by forming more stable and less reactive compounds in the presence of the *R. oligosporus* strain.

Compared to the control sample, the pre-treated ensiled elephant grass that was solid-state fermented, ensiled, and combined with *R. oligosporus* and urea had a significantly higher total phenolic content ($p<0.05$). Similar results were observed for other pre-treated ensiled elephant grass samples ($p<0.05$), but there was no significant difference in flavonoid concentration between the pre-treated ensiled elephant grass and control samples ($p>0.05$). However, the pre-treated ensiled elephant grass that was solid-state fermented and ensiled with *R. oligosporus* and urea had a significantly higher total phenolic content ($p<0.05$) than the sample that was only solid-state fermented and ensiled. This is consistent with previous research that has demonstrated the ability of phenolic compounds to quench oxygen radicals (de Siqueira et al., 2019; Liu et al., 2022). The polyphenolics in the fermented media likely served as effective electron and hydrogen atom donors, preventing the chain reaction of free radicals by converting them to more stable molecules (Egbune et al., 2022b).

The findings of the study are in agreement with earlier research on fermented seeds, which showed that fermentation increased the phenolic content of the seeds (Osete-Alcaraz et al., 2019; Inada et al., 2020 Santos et al., 2021). Phenolic compounds are typically bound to sugar, reducing their bioavailability to the body. During fermentation, proteolytic enzymes break down phenolic compounds into soluble-free phenols and other less complex but more physiologically active phenols that are readily absorbed (Gabriele and Pucci 2022; Rasera et al., 2023). The study also found that the phenolic concentration decreased as fermentation time

increased. This is likely due to the diffusion of phenolics in cell liquids and the oxidation of disseminated phenolics by polyphenol oxidase, as reported by Lasekan and Shabnam (2013).

The study found that birds fed diets comprising solid state fermented elephant grass, ensiled elephant grass with *R. oligosporus*, and ensiled elephant grass with *R. oligosporus* and urea did not exhibit any significant differences in weight gain when 25% of their maize diet was replaced. However, birds on other diets experienced significant weight loss. The biochemistry behind these results can be attributed to the nutritional composition of the diets. Ensilage is a method of preserving forages that involves anaerobic fermentation, which can improve the nutrient profile of the feed (Wilkinson and Rinne 2018; Egbune et al., 2021b). The addition of *R. oligosporus* to the ensiled elephant grass can further enhance its nutritional quality by breaking down antinutrients and increasing protein content (Egbune et al., 2023; Egbune, and Tonukari, 2023). Solid state fermentation of elephant grass also has the potential to improve its nutritional value by breaking down fiber and increasing the availability of nutrients (Debnath et al., 2022; Anigboro et al., 2022). Urea is a source of non-protein nitrogen that can be used to supplement low-protein feeds and improve the nitrogen balance in the diet (Lopes et al., 2020). Therefore, the diets containing these forages were able to provide the necessary nutrients for the birds to maintain their weight, resulting in no significant difference compared to the control diet. Overall, the use of forages in broiler feeding can have economic benefits, as they can reduce the volume and cost of feed used in production (Jørgensen et al., 2022). Additionally, the nutritional quality of the forages can contribute to maintaining weight and improving the overall health of the birds. The broiler chicks were able to consume and metabolize the experimental diets as well as the control feed with equal efficiency. These findings are consistent with previous studies conducted by Avwioroko et al. (2016), Tonukari et al. (2016), Egbune, and Tonukari, (2023), and Svihuset al. (2004). Using forages in broiler feeding could lead to a complete reduction in feed volume and cost, resulting in substantial cost savings.

Furthermore, the study examined the effects of the experimental diets on liver health by measuring serum alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) activities in the birds. The results showed no significant differences in these enzyme activities between birds fed the control diet and those fed the experimental diets, except for birds fed elephant grass and urea, whose ALT levels increased considerably (but not significantly) compared to the control. According to Koranteng et al. (2022), there was no difference in these enzyme activities when birds were fed fermented maize cob compared to the control. The ALT/AST activities observed in this study were within the normal range, and significant elevation of these enzyme activities could indicate liver damage, inflammation, or cell death. ALT and AST are involved in the metabolism of carbohydrates and proteins by transaminating key chemicals. Therefore, it can be concluded that the experimental diets were equally safe for the liver as the control diet, as there were no significant differences ($p>0.05$) in serum ALT and AST activities in birds fed the experimental diets compared to those fed the control diet.

Conclusion

Pre-treated and ensiled elephant grass can be a valuable ingredient in animal feed production, thanks to its significant increase in protein content and antioxidant activity. This study highlights the potential usefulness of mannanase in the animal feed industry, particularly for incorporating elephant grass into feed formulations. The results of the study suggest that adding pre-treated ensiled elephant grass to broiler starter diet does not have any adverse effects on blood biochemical profiles.

References

Anigboro, A. A., Aganbi, E. and Tonukari, N. J. (2020). Solid state fermentation of maize (Zea mays) offal by Rhizopus oligosporus under acidic and basic conditions. *J. Sci. Res.*, *12*(4), 751-756.

Anigboro, A. A., Egbune, E. O., Akeghware, O., Evie, P., Samofordu, A. A. and Tonukari, N. J. (2022). Biochemical parameters of solid-state

fermented cocoyam (Colocasia esculenta) using Rhizopus oligosporus at different inoculum sizes. *Niger. J. Biotechnol.*, *39*(1), 68-74.

Araujo, A. and Ward, O. P. (1990). Hemicellulases of bacillus species: preliminary comparative studies on production and properties of mannanases and galactanases. *J. Appl. Bacteriol.* 68 :253–261.

Avwioroko, O. J., Anigboro., A. A. and Tonukari, N. J. (2016). Biotechnological Application of Cassava-Degrading Fungal (CDF) Amylase in Broiler Feed Formulation. *Br. Biotechnol. J.* 10(1): 1-12.

Bakare, A. G., Kour, G., Akter, M. and Iji, P. A. (2020). Impact of climate change on sustainable livestock production and existence of wildlife and marine species in the South Pacific island countries: a review. *Int. J. Biometeorol.* 64: 1409–1421.

Baniwal, P., Mehra, R., Kumar, N., Sharma, S. and Kumar, S. (2021). Cereals: Functional constituents and its health benefits. *J. Pharm. Innov. 10*(2), 343-349.

Benzie, I. F. and Strain, J. J. (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power". The FRAP assay. Anal. Biochem. 239, 70–76.

Budhwar, S., Chakraborty, M., Sethi, K. and Chatterjee, A. (2020). Antidiabetic properties of rice and wheat bran—A review. *J. Food Biochem. 44*(10), e13424.

Chandel, A. K., Forte, M. B. S., Gonçalves, I. S., Milessi, T. S., Arruda, P. V., Carvalho, W.0 and Mussatto, S. I. (2021). Brazilian biorefineries from second generation biomass: critical insights from industry and future perspectives. *Biofuels Bioprod. Bioref.* 15:1190–1208.

de Siqueira, F. C., Leitão, D. D. S. T. C., Mercadante, A. Z., Chiste, R. C. and Lopes, A. S. (2019). Profile of phenolic compounds and carotenoids of Arrabidaea chica leaves and the in vitro singlet oxygen quenching capacity of their hydrophilic extract. *Int. Food Res. J. 126*, 108597.

Debnath, B., Duarah, P., Haldar, D. and Purkait, M. K. (2022). Improving the properties of corn starch films for application as packaging material via reinforcement with microcrystalline cellulose synthesized from elephant grass. *Food Packag. Shelf Life. 34*, 100937.

Egbune, E. O. and Tonukari, N. J. (2023). Fermented mixture of cassava roots and palm kernel cake can substitute for maize in poultry feed formulation. *Afr. J. Biochem. Res*. 17(1), 1-8

Egbune, E. O., Aganbi, E., Anigboro, A. A., Ezedom, T., Onojakpor, O., Amata, A. I. and Tonukari, N. J. (2023). Biochemical characterization of solid-state fermented cassava roots (ManihotesculentaCrantz) and its application in broiler feed formulation. *World J. Microbiol. Biotechnol. 39*(2), 1-12.

Egbune, E. O., Avwioroko, O. J., Anigboro, A. A., Aganbi, E., Amata, A. I. and Tonukari, N. J. (2022). Characterization of a surfactant-stable α-amylase produced by solid-state fermentation of cassava (*Manihot esculenta* Crantz) tubers using Rhizopus oligosporus: Kinetics, thermal inactivation thermodynamics and potential application in laundry industries. *Biocatal. Agric. Biotechnol. 39*, 102290.

Egbune, E. O., Eze, E., Edwards, R. A., Ezedom, T. and Tonukari, N. J. (2021a). Enhancement of the nutritional value of elephant grass (Pennisetum purpureum Schum.) for use as animal feeds and for xylanase production. *Niger. J. Sci. Envir*. *19*(2).

Egbune, E. O., Ezedom, T. and Nyerhovwo, J. T. (2021b). Inclusion of a locally produced animal performance supplement in broilers' ration. *Niger. J. Sci. Envir*. *19*(2).

Egbune, E. O., Ezedom, T., Anigboro, A. A., Aganbi, E., Amata, A. I. and Tonukari, N. J. (2022b). Antioxidants and antigenotoxic properties of Rhizopus oligosporus fermented cassava (Manihot esculenta Crantz). *Afr. J. Biochem. Res.,* 16(3), 39-46.

Ezedom, T., Egbune, E., Ehikordi, M., Ezeugo, N., Eledu, F., Esiete, J. and Tonukari, N. (2022). Biochemical evaluation of autoclaved and solid state fermented tropical pasture grasses. *J.*

Agricul. Biotechnol. Sustain. Develop. 14(2), 24-32.

Gabriele, M. and Pucci, L. (2022). Fermentation and germination as a way to improve cereals antioxidant and antiinflammatory properties. In *Current Advances for Development of Functional Foods Modulating Inflammation and Oxidative Stress* (pp. 477-497). Academic Press.

Hatano, T., Kagawa, H., Yasuhara, T. and Okuda, T. (1988). Two New Flavonoids and Other Constituents in Licore Root: Their Relative Astringency and Radical Scavenging Affects. C*hem. Pharmaceut. Bull.* 36, 2090- 2097

Inada, K. O. P., Silva, T. B. R., Lobo, L. A., Domingues, R. M. C. P., Perrone, D. and Monteiro, M. (2020). Bioaccessibility of phenolic compounds of jaboticaba (Plinia jaboticaba) peel and seed after simulated gastrointestinal digestion and gut microbiota fermentation. *J. Function. Foods*. *67*, 103851.

Jia, Z., Tang, M. and Wu, J. (1999). The determination of flavonoid contents of murlberry and their scavenging effects on superoxide radicals. *Food Chem.* 64, 555-559.

Jogawat, A., Yadav, B., Lakra, N., Singh, A. K. and Narayan, O. P. (2021). Crosstalk between phytohormones and secondary metabolites in the drought stress tolerance of crop plants: a review. *Physiologia Plantarum*, *172*(2), 1106-1132.

Jørgensen, U., Jensen, S. K. and Ambye-Jensen, M. (2022).Coupling the benefits of grassland crops and green biorefining to produce protein, materials and services for the green transition. *Grass Forage Sci*.

Kebede, E. (2020). Grain legumes production and productivity in Ethiopian smallholder agricultural system, contribution to livelihoods and the way forward. *Cogent food agric*., 6(1), 1722353.

Koranteng, A. A., Gbogbo, K. A., Adjei-Mensah, B., Bouassi, T., Aïna, C. T. F., Glago, J. and Kokou, T. (2022). Impact of palm kernel cake with or without multi-blend enzyme on the growth performance and carcass traits of Sasso broilers. *Int. J. Vet. Sci. Med. 10*(1), 80-89.

Lasekan, O.O. and Shabman, M. (2013). Effects of traditional fermentation as pretreatment to decrease the anti-nutritional properties of rambutan seed (Naphelium lappaceum L). International Conference on Food and Agricultural Science at 2013/ACSIT press. Singapore. Doi:107763/IPCBEE.

Liu, G., Zhu, W., Zhang, J., Song, D., Zhuang, L., Ma, Q. and Xu, X. (2022). Antioxidant capacity of phenolic compounds separated from tea seed oil in vitro and in vivo. *Food Chem. 371*, 131122.

Londoño-Hernandez, L., Bolivar, G. and Ramírez, T. C. (2018). Effect of solid state fermentation with Rhizopus oryzae on biochemical and structural characteristics of sorghum (Sorghum bicolor (L.) Moench). International Journal of Food and Fermentation Technology, 8(1), 27-36.

Lopes, A.S.M., de Oliveira, J.S., Santos, E.M., Medeiros, A.N., Givisiez, P.E.N., Lemos, M.L.P. and Oliveira, C.J.B. (2020). Goats fed with non-protein nitrogen: ruminal bacterial community and ruminal fermentation, intake, digestibility and nitrogen balance. *J. Agricul. Sci. 158*(8-9), 781-790.

Makini, F.W., Mose, L.O., Kamawu, G.K., Mulinge, W.W., Salasya, B., Makelo, M.N., Nyambati, E.M. and Fatunbi, A.O. (2019). Innovation opportunities in dairy livestock in Kenya. Guide book. *Forum for Agricultural Research in Africa*

Michalk, D.L., Kemp, D.R., Badgery, W.B., Wu, J., Zhang, Y. and Thomassin, P.J. (2018). Sustainability and future food security - A global perspective for livestock production. *Land Degradation* and *Development*.

Miller, G.L. (1959) Use of the Dinitrosalicylic Acid Reagent for the Determination of Reducing Sugar. *Anal. Chem.* 31, 426-428

Mutwedu, V.B., Manyawu, G.J., Lukuyu, M.N. and Bacigale, S. (2020). Fodder production manual for extension staff and farmers in South Kivu and Tanganyika Provinces of the Democratic Republic of the Congo. ILRI Manual 37. Nairobi, Kenya: International Livestock Research Institute (ILRI).

Naah, J.-B. S. N. and Braun, B. (2019). Local agro-pastoralists' perspectives on forage species diversity, habitat distributions, abundance trends and ecological drivers for sustainable livestock production in West Africa. *Sci. Rep.* 9:1707: 1-9.

Ndego, A., Ezedom, T., Egbune, E. O. and Tonukari, N. (2023). Biochemical characterization of solid state fermented maize cob (Zea mays) using Rhizopus oligosporusand its application in poultry feed production. *Int. J. Recycl. Org. 12*(2), 235-246.

Negawo, A., Teshome, A., Kumar, A., Hanson, J. and Jones, C. (2017). Opportunities for Napier Grass (*Pennisetum purpureum*) Improvement using molecular genetics. *Agronomy* 7(2), 28:1-21.

Ojo, I., Apiamu, A., Egbune, E. O. and Tonukari, N. J. (2022). Biochemical characterization of solid-state fermented cassava stem (Manihot esculenta Crantz-MEC) and its application in poultry feed formulation. *Appl. Biochem. Biotechnol. 194*(6), 2620-2631.

Olukomaiya, O.O., Adiamo, O.Q., Chrishanthi Fernando, W., Mereddy, R., Li, X. and Sultanbawa, Y. (2020). Effect of solid-state fermentation on proximate composition, anti-nutritional factor, microbiological and functional properties of lupin flour. *Food Chem.* 315:126238.

Osete-Alcaraz, A., Bautista-Ortín, A. B., Ortega-Regules, A. E. and Gómez-Plaza, E. (2019). Combined use of pectolytic enzymes and ultrasounds for improving the extraction of phenolic compounds during vinification. *Food Bioprocess Technol. 12*, 1330-1339.

Qin, P., Wang, T. and Luo, Y. (2022). A review on plant-based proteins from soybean: Health benefits and soy product development. *J. Agric. Food Res. 7*, 100265.

Rasera, G.B., de Camargo, A.C. and de Castro, R.J.S. (2023). Bioaccessibility of phenolic compounds using the standardized INFOGEST protocol: A narrative review. *Compr. Rev. Food Sci. Food Saf. 22*(1), 260-286.

Santos, T.R., Feitosa, P.R., Gualberto, N.C., Narain, N. and Santana, L. C. (2021). Improvement of bioactive compounds content in granadilla (Passiflora ligularis) seeds after solid-state fermentation. *Food Sci. Technol. Inter. 27*(3), 234-241.

Sharma, P., Jha, A.B., Dubey, R.S. and Pessarakli, M. (2021). Reactive oxygen species generation, hazards, and defense mechanisms in plants under environmental (abiotic and biotic) stress conditions. *Handbook of plant and crop physiology*, 617-658.

Simeão RM, Resende M.D.V., Alves R.S., Pessoa-Filho M., Azevedo A.L.S., Jones C.S., Pereira J.F. and Machado, J.C. (2021) Genomic selection in tropical forage grasses: current status and future applications. *Front. Plant Sci.* 12:665195.

Singleton, V.L. and Rossi, J.A. (1965) Colorimetry of Total Phenolics with Phosphomolybdic-Phosphotungstic Acid Reagents. *Am. J. Enol. Vitic.*16, 144-158.

Tonukari, N. J., Oliseneku, E. E., Avwioroko, O. J., Aganbi, E., Orororo, O.C. and Anigboro, A.A. (2016). A novel pig feed formulation containing Aspergillus niger CSA35 pretreated-cassava peels and its effect on growth and selected biochemical parameters of pigs. *Afr. J. Biotechnol. 15*(19), 776-785.

Weishaupt, A., Ekardt, F., Garske, B., Stubenrauch, J. and Wieding, J. (2020). Land Use, Livestock, Quantity Governance, and Economic Instruments—Sustainability Beyond Big Livestock Herds and Fossil Fuels.

Wilkinson, J. M. and Rinne, M. (2018).Highlights of progress in silage conservation and future perspectives. *Grass Forage Sci. 73*(1), 40-52.