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IMPACT OF AUTOCLAVING AND SOLID-STATE FERMENTATION ON THE BIOCHEMICAL COMPOSITION OF TROPICAL PASTURE GRASSES

[1]Bahati M. Nyangoma

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Abstract

Forage resources are crucial for livestock production worldwide, yet they face significant challenges related to availability, quality, and productivity. Various feed additives have been introduced to commercial livestock feed to enhance its quality, including antioxidants that prevent lipid peroxidation and oxidative rancidity. Foods rich in polyunsaturated fatty acids are particularly prone to lipid peroxidation, which can destroy essential nutrients and compromise the sensory qualities of the feed. The inclusion of exogenous antioxidants in livestock feeds has been shown to mitigate these issues, preserving nutrient integrity and improving feed quality. This study conducts a biochemical evaluation of autoclaved and solid-state fermented tropical pasture grasses, examining their potential as effective feed resources with enhanced nutritional and preservative properties.

INTRODUCTION

Forage resources for livestock production are quite feed additives have been added to commercial feed to enormous worldwide, but they face challenges related to improve the quality of livestock feed. This includes the availability, quality and productivity of pastures. Different use of antioxidants, which help in the prevention of lipid peroxidation and oxidative rancidity. It has been shown that foods rich in polyunsaturated fatty acids are highly susceptible to lipid peroxidation (Decker et al., 2012). Thus, the inclusion of exogenous antioxidants in livestock feeds aids in the prevention of the destruction of essential nutrients (Calabotta and Shermer, 1985) as well as the preservation of the feed's sensory qualities (Halliwell and Gutteridge, 1999; Surai, 2007; Kalam et al., 2012).

Natural antioxidants obtained from plants' phytochemicals have been shown to be capable of inhibiting the generation of reactive oxygen species (Moukette et al., 2015). These compounds are phenolic in nature and consist of, among others, flavonoids, phenolic acids, tocopherols and carotenoids (Dubey et al., 2014). According to Balasundram et al. (2006), phenolic compounds are major determinants of food antioxidant potential.

[1] Department of Agriculture and Biochemistry, Tanzania.

Flavonoids, which are secondary plant metabolites, consist of flavanols, anthocyanidins, flavones, and isoflavones (Hollman, 2004). The consumption of dietary antioxidants in adequate quantity has been shown to protect against free radical activity. It also plays a significant role in disease prevention (Alam et al., 2013).

Antioxidants have been defined as substances that when present in feeds and foods at a concentration lower than that of an oxidizable substrate will significantly interrupt or avert the oxidation of the substrate (Halliwell and Gutteridge, 1999). According to Hilton (1989) and Decker (1998), substances that exhibit antioxidative capacity include oxygen scavengers, deactivators of peroxides and other reactive oxygen species, free radical scavengers, quenchers of secondary lipid oxidation products that produce rancid odors and metalchelates. Antioxidants used in animal feeds can be broadly categorized into natural and synthetic ones. However, the excessive use of synthetic antioxidants may be implicated in carcinogenic and/or mutagenic effects on consumers (Fellenberg and Speisky, 2006). Thus, nutritionists are now searching for the natural antioxidants for animal feeds.

Grasses are monocotyledonous plants that belong to the Poaceae or Gramineae family (Dashora and Gosavi, 2013). They are widely spread and abundant (Hubbard, 1954). They can be classified as annual, biennial or perennial. Grasses grow in many areas, but some are restricted to particular areas due to climatic and environmental conditions (Milton, 2004). They have different adaptability to many locations and tenacity. Grass constitutes the nutritional basis for most livestock. Whether domesticated or wildlife, grass is the mainstay of some livestock. In order to improve the nutritional quality of grass, various processing techniques have been applied (Simeão et al., 2021; Chandel et al., 2021; Egbune et al., 2021b). Solid state fermentation involves the growth of microorganisms on substrates with limited water content (Dulf et al., 2017). Studies have shown that various agricultural by-products can be enhanced by solid state fermentation (Bennett and Yang, 2012; Dulf et al., 2016; Jin et al., 2016). Solid state fermentation has been widely used in the feedstock industry to promote nutrient utilization (Chi and Cho, 2016; Wang et al., 2018a; Egbune et al., 2021d). An autoclave is an application, which is generally used for heat treatments. When this application is used on cereals and other plant-based foods, it activates the phytase enzyme as well as increases acidity (Ertop and Bektaş 2018).

The demand for animal protein for human nutrition in the developing world is still increasing, particularly for chicken products, and the cost of livestock feed concentrates is rising (Naylor et al., 2021; Egbune et al., 2021b). As a result, in order to satisfy the nutritional needs of chickens, new low-cost feed alternatives must be identified. Forages used as feed for monogastric animals, particularly poultry, help to increase the sustainability of animal production in farming systems (Lüscher et al., 2014) Plants generate a wide range of anti-nutritional chemicals, many of which have been discovered and described. Polyphenols, cyanogenic glycosides, alkaloids, saponins, steroids, toxic proteins and amino acids, non-protein amino acids, phytohemagglutinins, triterpenes, and oxalic acid are the most prevalent main groupings that are either poisonous or anti-nutritive (Ali et al., 2022). Secondary compounds can have a significant impact on the nutritional content and feeding value of both temperate and tropical forages and these effects can be advantageous in certain cases while being harmful in others. However, forage plants may be effectively treated to improve palatability, intake, and digestibility, as well as to save, detoxify, or concentrate nutrients. This present study aims to evaluate the efficacy of two pretreatment techniques for processing common grasses consumed by ruminants.

MATERIALS AND METHODS

Collection of grasses and treatments

Palisade grass (*Urochloa brizantha*), Biscuit grass (*Paspalum vaginatum*), Giant star grass (*Cynodon plectostachyus*), and Goose grass (*Eleusine indica*) were harvested from Delta State University, Abraka, Delta state and was identified and authenticated in the Department of Botany, University of Benin, Edo State, Nigeria with Voucher number: UBH-P288, UBH-P151, UBH-C334 and UBHP398, respectively. The samples were

pulverized using a commercial grinding machine (SM-1 Retsch GmbH, 5667 HAAN) and stored at room temperature. *Rhizopus oligosporus* strains for solid state fermentation were obtained from the Harmony Path, Ltd. laboratory located at Songhai in Amukpe, Sapele, Delta State. Solid state fermentation was carried out according to the method described by Ofuya and Nwajiuba (1990), at pH 6 in bio-fermenter using 50 mM phosphate buffers at room temperature for 72 h. One gram (1 g) of each grass sample (1.4×10^2 CFU) was homogenized in 10 ml of prepared phosphate at pH 6 in a bio fermenter. 10 g of the ground palisade grass was used in the homogenization step and allowed to ferment for a 72 h period at room temperature. The autoclaving of the grasses was done at 121°C for 30 min.

Unfermented control samples (containing dried and grounded palisade grass, devoid of any presence of molds, with buffer only, and without any cells) were prepared alongside the test samples. After fermentation and autoclaving, 6 g of the mixtures were measured, and 40 ml of distilled water was added and homogenized using mortar r and pestle. 10 ml of the mixture was collected into a test tube and centrifuged for 10 min to get the crude extract. This supernatant (crude extract) was used as the crude extract or sample for the various assays which were carried out in triplicates.

Biochemical assays

The method of Gornall et al. (1949) was employed in assaying for total soluble proteins using bovine serum albumin as the standard. pH of the sample was measured using a Mettler Toledo pH meter. The colorimetric estimation of reducing sugars was done using the method of Miller (1959). Glucose level was assayed as described in the Randox glucose kit following the manufacturer's (Randox Laboratories Ltd, County Antrimm BT29 4QY, United Kingdom) instructions. Antioxidant inhibition of 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical, total phenol and flavonoid contents were determined based on the procedures of Hatano et al. (1988), Singleton and Rossi (1965), and Jia et al. (1999), respectively. One unit of xylanase activity (U) was defined as the amount of the enzyme required to liberate 1 µmol of xylose per min under the assay conditions. The yields were expressed as U per gram dry substrate.

Statistical analysis

Data obtained were expressed as mean ± standard deviation and analyzed using analysis of variance (ANOVA). Comparism of the various group means was done using Fischer test of least significance (LSD). P-values less than 0.05 ($p<0.05$).is considered significant at 95% confidence level.

RESULTS AND DISCUSSION

Changes in pH values of Palisade, Biscuit, Giant star and Goose grasses after the various treatments are shown in Figure 1.

Results obtained showed a decreased in pH values for autoclaved grasses while the solid state fermented grasses showed an increase in pH values. The initial pH of solid state fermented palisade grass increased from 6.0 to 7.0 at the end of the 72 h fermentation period for all grasses, which is an indication of the favorable state of solid state fermentation of grasses. Most microorganisms function optimally at neutrality, while a few require an alkaline environment. Inhibition of microbial activities has been recorded for acidic pH (Aganbi et al., 2020).

Thus, a shift in the pH during the fermentation process could have been contributed by the strong proteolysis of the fungi *R. oligosporus*. This is in accordance with the report of Owens et al. (1997), who reported a rise in pH value from 4.5 to 8.5 in soybean fermentation. This was associated with a strong ammoniacal odour due to the hydrolysis of seed protein and resultant amino acid metabolism.

The outcomes of the determination of the levels of proteins, glucose and reducing sugars in the various pretreatments of the grasses are shown in Figures 2 to 4. There was a substantial increase ($p<0.05$) in soluble protein content from 33.1±0.8 to 64.7±0.9 mg g^{-1} of biscuit grass fermented with *R. oligosporus*, while there was

a total decline in the levels of soluble protein in other pretreatments. The increase in the level of soluble protein of the solid state fermented grasses could be attributed to the production of extracellular enzymes such as hemicellulase, cellulase, and lignases to make use of carbon sources in the substrate.

These extracellular enzymes are protein-based and could have contributed to the protein biomass of the substrate (Sharma et al., 2017; Akassou and Groleau, 2019; Ong and Lee, 2021). According to Elyas et al. (2002), this could also be as a result of the synthesis of proteins from metabolic intermediates during their growth cycles. It could also be as a result of a change in dry matter content by the action of the fermenting microorganisms (Cai et al., 2019; Egbune et al., 2021c). The results of the present study show a general increase in the level of glucose in the solid state fermented grasses when compared to the control. There was a substantial rise ($p<0.05$) in glucose concentration of solid state fermented biscuit grass (54.6±3.3 mg g^{-1}) when compared to the control (32.4±1.6 mg g^{-1}). Anigboro et al. (2020) and Da Silva et al. (2019) have also reported increase in glucose level upon *R. oligosporus* fermentation of maize (*Zea mays*) offal. The potential hydrolysis of complex carbohydrates such as sucrose, pectin, cellulose, etc. has been shown to increase the level of glucose in a substrate (Marques et al., 2016; Murthy and Naidu, 2011).

The results of the determination of reducing sugar concentration in the different pretreatments of the five different grasses are shown in Figure 4. Fermented grasses showed a significant level of reducing sugar compared to the control. This could be as a result of the substrate being degraded and used as a carbon source. Similar observations have been recorded by Kolo et al. (2020).

The results of the determination of total phenolic content and total flavonoid content in the different pretreatments of the different grasses are shown in Figures 5 and 6. There was a significant increase ($p < 0.05$) in total phenolic content from untreated goose grass (Control) (26.5 ± 0.9 mg g^{-1}) to 77.6 ± 5.4 mg g^{-1} in solid state fermented goose grass using *R. oligosporus*.

A general increase in the total phenolic content was observed across the various pretreatments for all grasses. An increase in the level of total phenolic content in all grasses could be attributed to the release of free total phenolic content from the bound state, thereby improving bioavailability by the proteolytic activities of fungi (Dey et al., 2016). Phenolic compounds have been shown to have antioxidant and anticancer activities (Balasundram et al., 2006). Solid-state fermentation has the potentials to increase the organoleptic and nutritional properties of food. It has also been shown to be capable of converting agro-industrial residues or plants into valuable phenolic compounds (Liu et al., 2017; Martins et al., 2011). The total flavonoid content was improved in the solid state fermented grasses (116.7 ± 1.4 µg/ml) when compared to the control. The highest level of total flavonoid content was obtained in all grasses in the solid state fermentation at 72 h fermentation. Phenolic compounds and flavonoids are well known antioxidants.

Figure 7 shows the free radical scavenging activities in the different pretreatments of the grasses as compared to the control. The result showed that grasses subjected to solid state fermentation had a higher level of free radical scavenging activity compared to the control. Observed changes in the free radical scavenging ability of the solid state fermented elephant grass have been attributed to the process of fermentation, which could have generated secondary metabolites (Martins et al., 2011) Fermentation also involves the structural breakdown of plant cell walls.

This could indirectly increase the levels of antioxidant compounds (Hur et al., 2014).

The activities of xylanase differ markedly with the different pretreatments of the different grasses. However, generally, there was a significant increase ($p < 0.05$) in xylanase activity in solid state fermented grasses when compared to the control. Thus, in the absence of fermentation, that is, in control, the levels of nitrogen could be too low to support growth and enzyme production and growth (Tai et al., 2019; Egbune et al., 2021a).

Xylanase is the enzyme that converts the linear polysaccharide xylan to xylose (Malgas and Pletschke, 2019; Javed et al., 2019). This is a major component of plant cell walls. Thus, this enzyme plays an important part in the breakdown of plant materials into useable nutrients by microorganisms that rely on plants for nourishment, such as grass.

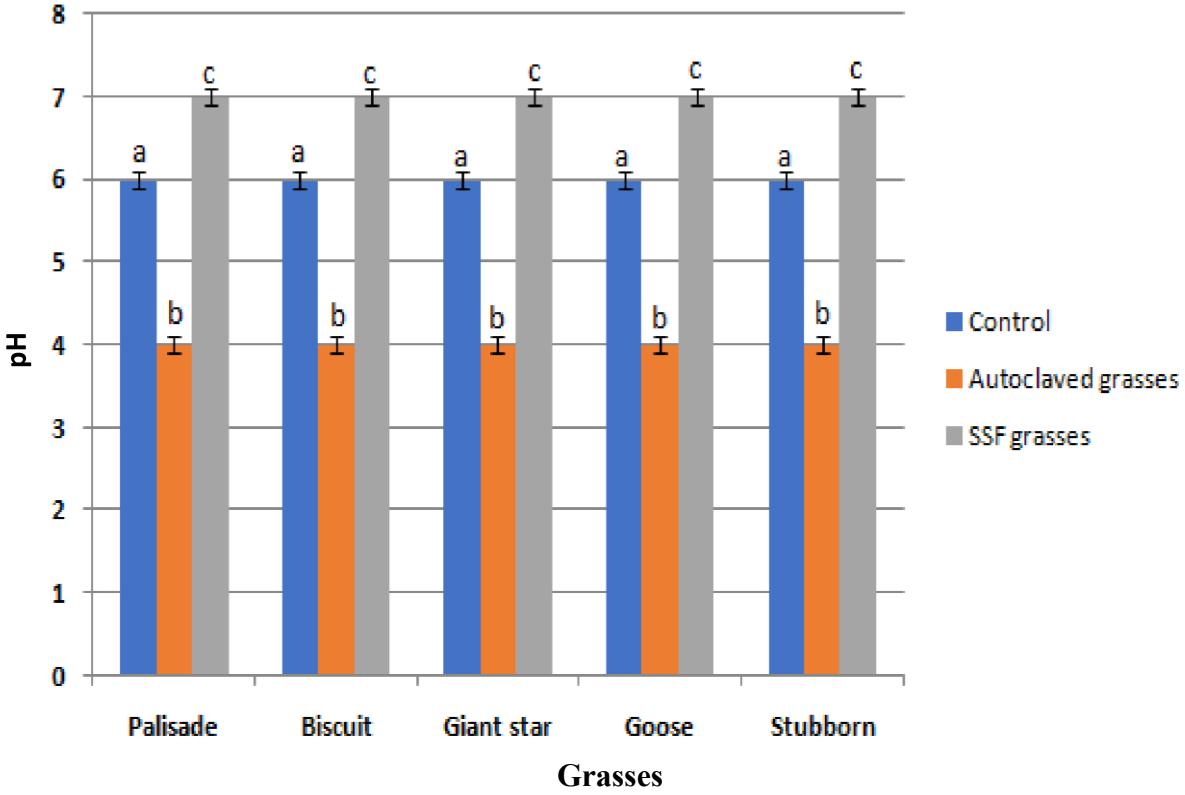

Figure 1. pH changes in the different pretreatments of five different pasture grasses. Bars with different superscript differ significantly at ($p < 0.05$). Source: Authors

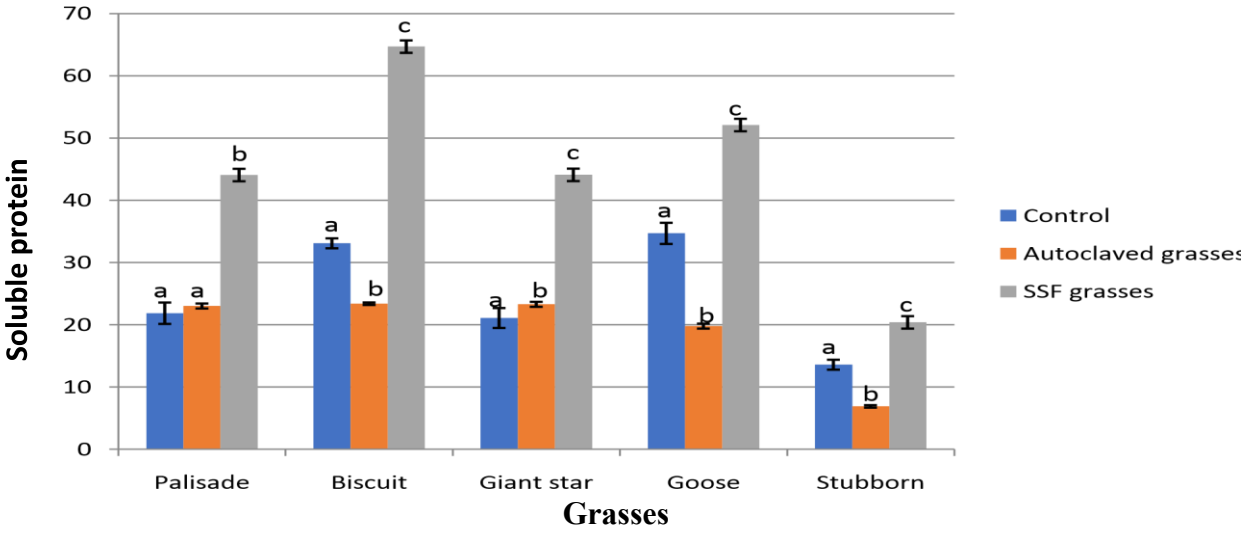

Figure 2. Estimation of the level of soluble protein in five different pasture grasses exposed to experimental treatments. Bars with different superscript differ significantly at ($p < 0.05$).
Source: Authors

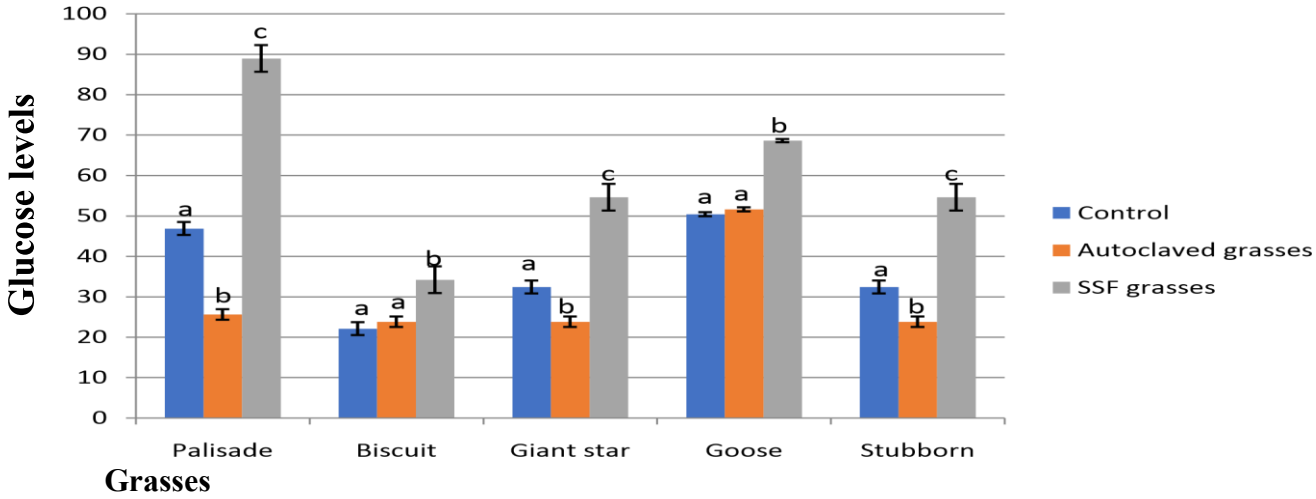

Figure 3. Estimation of glucose level in five different pasture grasses exposed to experimental treatments. Bars with different superscript differ significantly at ($p < 0.05$). Source: Authors

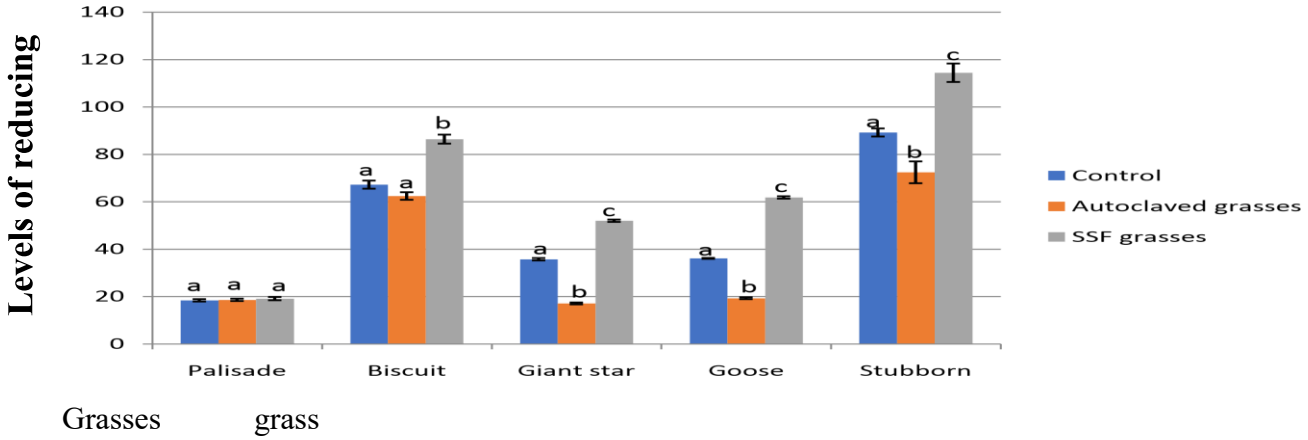

Figure 4. Estimation of the levels of reducing sugar in five different pasture grasses exposed to experimental treatments. Bars with different superscript differ significantly at ($p < 0.05$).
Source: Authors

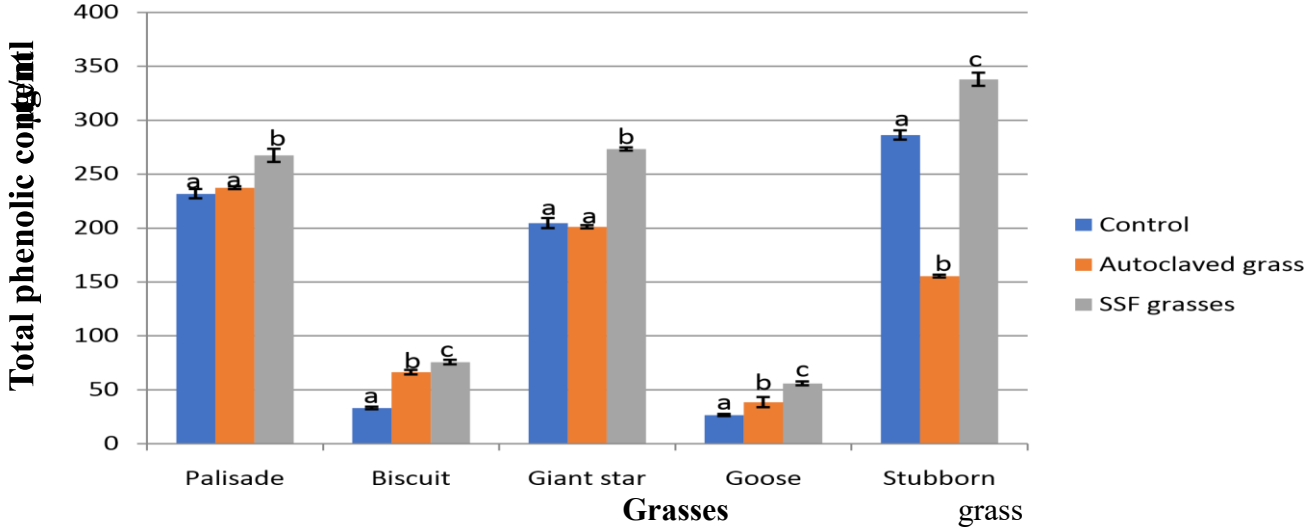

Figure 5. Total phenolic content of five different pasture grasses exposed to experimental treatments. Bars with different superscript differ significantly at ($p < 0.05$). Source: Authors

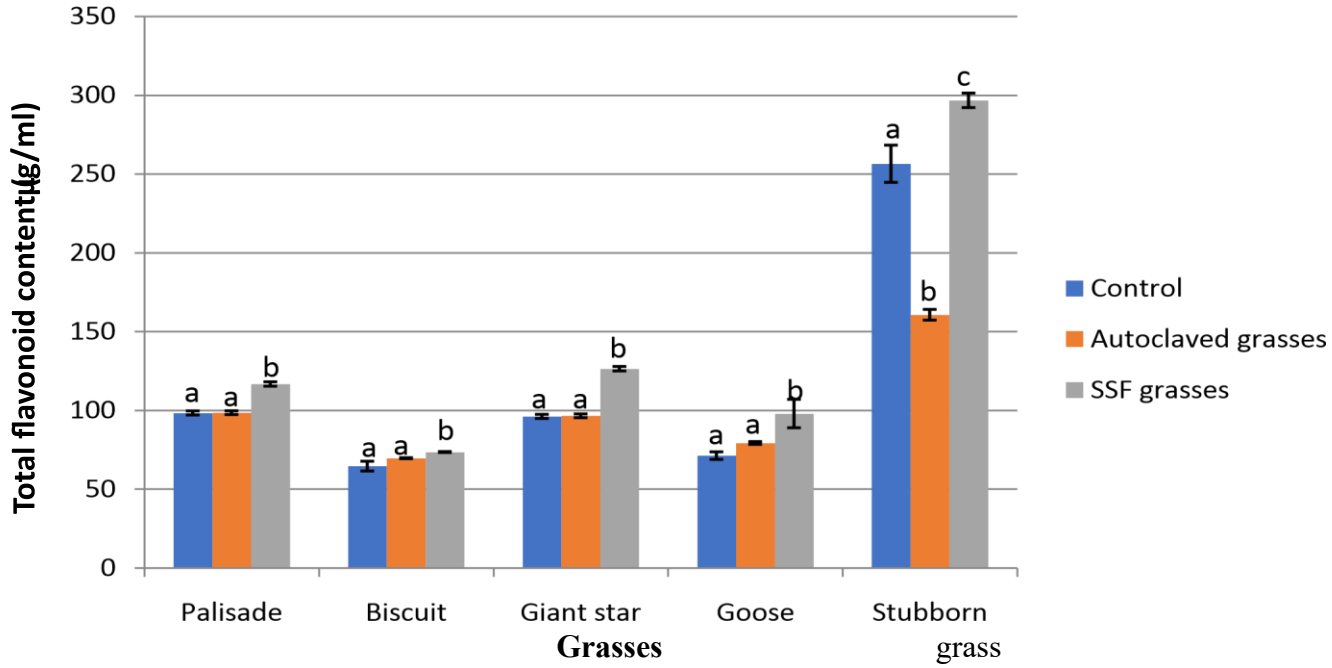

Figure 6. Total flavonoid content of five different pasture grasses exposed to experimental treatments. Bars with different superscript differ significantly at ($p < 0.05$).

Source: Authors

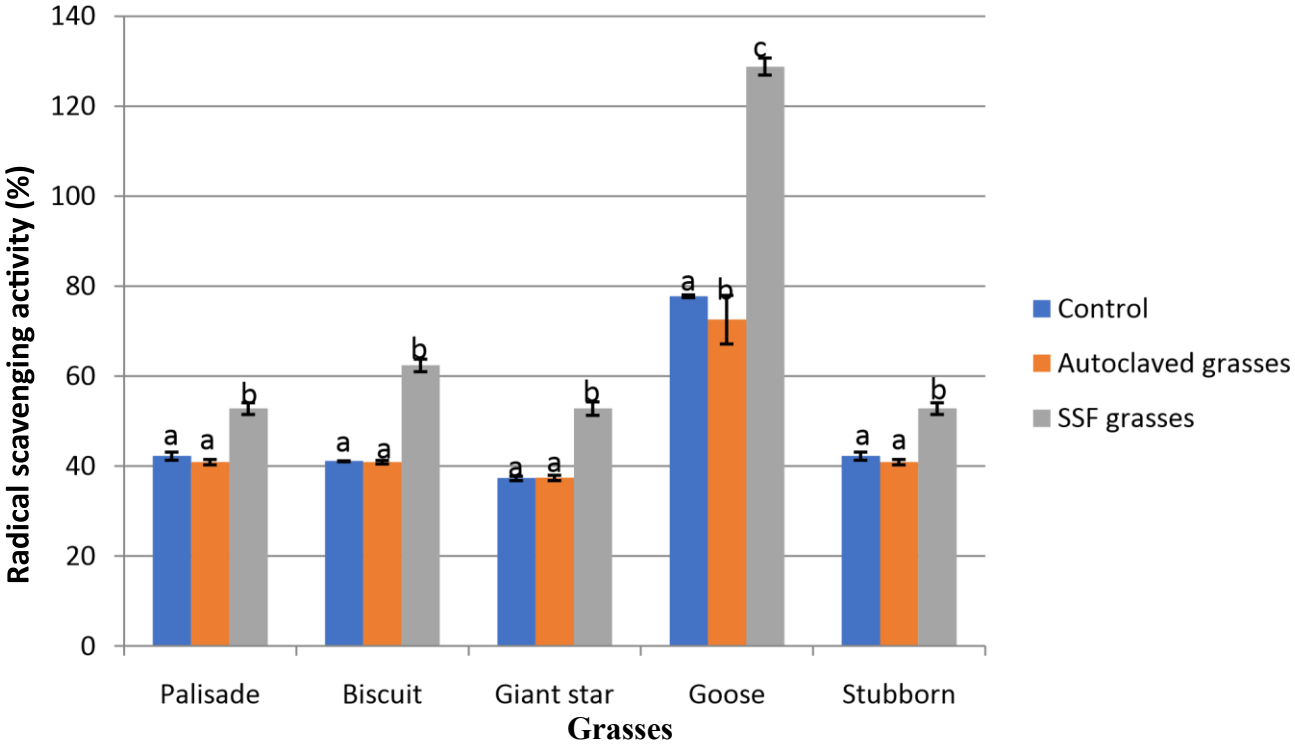

Figure 7. Radical scavenging activities of five different pasture grasses exposed to experimental treatments. Bars with different superscript differ significantly at ($p < 0.05$). Source: Authors

Conclusion

Conclusively, the present study has shown that solid state fermentation could improve the antioxidant and nutritive values (proteins, glucose and reducing sugar) of different grasses. Thus, solid state fermentation could be applied industrially to reduce the availability of quality feed for livestock. The ability of solid state fermented elephant grass to enhance the level of production of xylanase further helps in ensuring the bioavailability of nutrients in the plants for absorption. The observation from the preceding study is significant to accelerating forage optimization using solid-state bio-processing approach. The functional and nutritional qualities of the solid-state fermented forages demonstrated that they might be useful components in feed compositions.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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