Enriched Nutrients Of Napier Grass Using *Aspergillus* Spp. Through Fermentation

Mohammad Mijanur Rahman[1,2], Nur Farizah Mohamed Saberi[1], Mst. Laila Naher[1], Nafiatul Umami[3], Nor Dini Rusli[1,2], Khairiyah Mat[1,2], Guillermo Téllez-Isaías[4], Albaris B. Tahiluddin[5], Khang Wen Goh[6], Lee Seong Wei[1,2] and Zulhisyam Abdul Kari[1,2*]

[1]*Faculty of Agro Based Industry, Universiti Malaysia Kelantan, 17600 Jeli, Kelantan, Malaysia
[2]Advanced Livestock and Aquaculture Research Group, Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, Jeli Campus, 17600 Jeli, Kelantan, Malaysia
[3]Department of Animal Science, Faculty of Animal Science, Universitas Gadjah Mada, 55281, Yogyakarta, Indonesia
[4]Department of Poultry science, University of Arkansas, Fayetteville, AR 72701, USA
[5]College of Fisheries, Mindanao State University-Tawi-Tawi College of Technology and Oceanography, Sanga-Sanga, Bongao, Tawi-Tawi 7500, Philippines
[6]Faculty of Data Science and Information Technology, INTI International University, 71800 Nilai, Malaysia.

***Corresponding Author:-** Zulhisyam Abdul Kari
*Faculty of Agro Based Industry, Universiti Malaysia Kelantan, 17600 Jeli, Kelantan, Malaysia

Abstract
Napier grass *(Pennisetum purpureum)* is widely used for ruminant feeding due to its high yield and low input management. Because of the low nutritive value of Napier grass, it is required to enhance it using additives (e.g., molasses and fungi) that can fulfil the nutrient requirement of ruminants. In this work, Napier grass was ensiled with or without fungus for 21 days: without fungus (T1), with *Aspergillus niger* (T2), and with *A. awamori* (T3), to investigate the influence of *Aspergillus* spp. on fermentation characteristics and nutritive values. The results revealed that the application of *Aspergillus* spp. increased ($p<0.05$) pH level. Lactic acid content did not differ ($p>0.05$) significantly between treatments. The ammonia nitrogen content was higher ($p<0.05$) in *A. niger* treated silage, followed by *A. awamori* treated silage and untreated silage. The ammonia nitrogen, crude protein (CP), dry matter (DM), nitrogen-free extract (NFE), and ether extract (EE) contents varied significantly ($p<0.05$) among the treatments, while the ash contents and crude fiber (CF) did not differ. The T2 silage showed the highest while the T1 silage showed the lowest as follows: DM (24.0% vs. 21.0%), CP (11.4% vs. 9.2%), CF (9.3% vs. 8.3%), and ash (9.4% vs. 6.3%) contents. The T2 silage showed the highest EE content (7.7%), while T3 showed the lowest (0.6%). The T1 silage showed the highest NFE content (74.7%), while the T2 silage showed the lowest (62.3%). The above findings suggested that the *A. niger* addition could enhance fermentation characteristics and nutritive values of silage.

Keywords: *Aspergillus niger*, *Aspergillus awamori*, fermentation quality, Napier grass, nutritional value

INTRODUCTION

Pennisetum purpureum, commonly called as Napier grass, is one of the major perennial equatorial forage grasses and is utilized as one of the major feeds for ruminants for various reasons, including low input management, great biomass yield (de Morais et al., 2009), easy to propagate, great potential re-growth (Lee et al., 2016), reduce soil erosion (Magcale-Macandog et al., 1998), immerse capability for cellulosic biofuel production (Tsai & Tsai, 2016), and resistant to numerous microorganisms (Van den Berg & Van Hamburg, 2015). However, its nutritional content is still low (7-9% crude protein and 7.5 MJ metabolizable energy/kg dry matter) and not adequate for the ruminant's nutrient requirement. To establish Napier grass as the primary consumption for ruminants, it has been suggested that a portion of the Napier grass requires to be replaced with great protein or energy feed to

enhance rumen digestibility and microbial activity (Gwayumba et al., 2002).

Nearly 70% of the total costs in animal production are contributed by feedstuffs. In feedstuff costs, protein sources represent a huge amount, and it is costly. To lessen the pressure on protein sources, there is a need for novel protein sources to be explored by animal nutritionists. Fungus *Aspergillus niger* is commonly found in various foods, such as nuts, grains, and spices, particularly in tropical and subtropical regions (Pitt and Hocking, 2009). In addition, A. *niger* can enhance the nutritive value of feeds, and it is desirable for solid-state fermentation owing to its rapid growth in a water-limited environment (Oriol et al., 1988).

Previous studies indicated that *A. niger* could also boost feed digestibility by generating enzymes such as protease lipase, xylanase, cellulase, and amylase (Oyeleke et al., 2012; Morgado et al., 2016). Furthermore, using *A. niger* in solid-state fermentation resulted in 30% to 80% excellent enzymatic activities compared with standard submerged fermentation (Madamwar et al., 1989). Thus, the inclusion of *Aspergillus* species in the solid-state fermentation of Napier grass might help in lengthening its shelf-life and enriching its nutritional value. Therefore, the present study aimed to investigate the effects of *A. niger and A. awamori* on the fermentation characteristics and nutritive values of Napier grass.

MATERIALS AND METHODS

Location and materials used in this study

This study was conducted at the Faculty of Agro Based Industry (FIAT), Universiti Malaysia Kelantan (UMK) during the period of July-December 2021. *A. niger* strain was obtained from Global Manufacturing Distribution Centre (located at 200 Cooper Avenue North, Saint Cloud, MN, 56303), cultured, and derived from ATCC® 6275TM in KWIK-STIK form in a lyophilized pellet, a reservoir of hydrating fluid and inoculating swab. *A. awamori* was subcultured from a previous agar plate that was obtained from FIAT, UMK. Re-growth Napier grass at approximately two months of plant maturity was harvested and chopped into about 2-3 cm in length manually from Agro Techno Park (ATP), UMK. Molasses was obtained from a local supplier.

Preparation of fungus culture

Aspergillus niger and *A. awamori* were incubated at the laboratory of FIAT, UMK, at room temperature for seven days following the agar plate technique (Güngör et al., 2017). The agar plate technique was done by taking a drop of the culture on the upper layer of nutrient agar, then using a loop wire to make a streaking. After that, it was incubated for another seven days at 40°C for a subculture to isolate the pure culture (organism) and then incubated again for another seven days until the fungus reached maturity (turn into green) (Naher et al., 2012). The spores were prepared for the experimental treatments. Using serial dilution method, the spores were prepared to estimate the fungus concentration by counting the number of colonies cultured. Next, the number of spores was counted using Hemocytometer according to the colony-forming unit (CFU/ml) (Güngör et al., 2017). The CFUs were used to count the appropriate amount of concentration of fungal cells in a test sample that was viable under the microscope. The concentration used was 10^6 spores ml^{-1} of the cultured fungus. *A. niger* and *A. awamori* were diluted with 10 ml of sterile distilled water and shaken well to dissolve each other to make the stock solution (Altop, 2019). Then, the stock solutions were inoculated with Napier grass silage.

Experimental design

Chopped Napier grass was fermented using molasses and fungus for 21 days. Three treatment silages were prepared with three replications for each treatment: (i) Napier grass ensiled with 5% molasses (T1), (ii) Napier grass ensiled with 5% molasses and *A. niger* (10 ml/kg silage; 10^6 spores/ml) (T2), and (iii) Napier grass ensiled with 5% molasses and *A. awamori* (10 ml/kg silage; 10^6 spores/ml) (T3). Mixed silages (about 500 gm for each replication) were placed into empty zip lock plastic, packed, airtight, sealed, and stored properly under anaerobic conditions to allow the fermentation process.

Chemical analysis

After ensiling for 21 days, the samples from each bag were taken and oven-dried for 48 hours at 60 °C. Then, the dried samples were ground and passed through a 1-millimeter meshed sieve for chemical analysis. The crude protein (CP), ether extract (EE), crude fiber (CF), nitrogen-free extract (NFE), and ash contents were measured following the method of AOAC (2000). The ammonia nitrogen (NH_3-N) was determined utilizing the Kjeldahl method (AOAC, 2000).

The pH value of the fermented silages was measured using a pH meter. Approximately 10 g of fresh fermented silage sample for each replication were placed in a beaker containing 50 ml distilled water for 30 minutes at room temperature. After 30 minutes, the pH value was recorded by placing the electrode pH meter in the silage sample. Lactic acid content was determined following the method of Amin et al. (2004). About 5 g of fresh fermented samples were weighed and placed into a beaker, and then 50 ml of distilled water was added. The sample was boiled to drive off CO_2 and cooled. The needed samples were filtered by using filter paper, and filtrates were collected. Then, five drops (5 ml) of 1% phenolphthalein were added to the filtrate. The filtrates were titrated with 0.1N NaOH until they changed into light pink or pale pink in colour. Total lactic acid content was expressed as % lactic acid.

Statistical analysis

Using SPSS software (version 23), all data were analyzed using a one-way analysis of variance (ANOVA). The Duncan Multiple Range Test was also utilized to differentiate between treatments at $p<0.05$.

RESULTS

Fermentation characteristics

The fermentation characteristics of Napier grass silages are presented in Table 1. The pH value of the T1 silage was significantly ($p<0.05$) lower (4.08) than the T2 (4.20) and T3 (4.17) silages, while no significant ($p>0.05$) difference was recorded in the pH value between T2 and T3 silages. Similarly, the NH_3–N contents in Napier grass silages remarkably ($p<0.05$) differed among the treatments. The T2 silage contained significantly ($p<0.05$) higher NH_3–N content (1.82%), followed by T3 (1.64%) and T1 (1.46%) silages. On the other hand, lactic acid contents were not varied ($p>0.05$) among the treatment silages, and its concentration in experimental silages ranged from 5.75 to 7.16% of the DM.

Table 1: Fermentation characteristics of Napier grass treated with or without *Aspergillus* sp.

Parameter	Treatments (mean ± standard deviation)			p-value
	T1	T2	T3	
pH	4.08 ± 0.15^{a}	4.20 ± 0.45^{b}	4.17 ± 0.20^{b}	0.008
Ammonia nitrogen (%)	1.46 ± 0.11^{a}	1.82 ± 0.83^{c}	1.64 ± 0.17^{b}	0.006
Lactic acid (%)	5.77 ± 0.50	7.16 ± 1.22	5.75 ± 0.64	0.142

^{abc} means with different superscripts in a row differ significantly ($p<0.05$). T1 = Napier grass silage treated with 5% molasses; T2 = Napier grass silage treated with 5% molasses and *Aspergillus niger* (10 ml/kg silage; 10^6 spores/ml); T3 = Napier grass silage treated with 5% molasses and *Aspergillus awamori* (10 ml/kg silage; 10^6 spores/ml).

Proximate components

The proximate components of experimental silages are shown in Table 2. Silage treated with *A. niger* showed significantly ($p<0.05$) higher DM content (24.0%), followed by silage treated with *A. awamori* (23.0%) and untreated silage (21.0%). The T2 silage contained significantly ($p<0.05$) higher CP and EE contents (11.4% and 7.7%) than T3 (10.2% and 0.6%) and T1 (9.2% and 1.5%) silages, respectively. There were remarkable ($p<0.05$) differences in CF content among the treatment silages. Table 2 shows that the silage treated with *A. niger* showed significantly ($p<0.05$) higher (29.0%) CF content, followed by silage treated with *A. awamori* (25.3%) and untreated silage (23.3%). However, no statistical ($p>0.05$) differences were detected between treatments in terms of ash content in Napier grass silages. In contrast, the NFE contents of experimental

silages varied significantly ($p<0.05$) among the treatments. The percentage of NFE of the T2 silage was significantly ($p<0.05$) lower (42.6%) than the T1 (59.7%) and T3 (55.5%) silages, while no significant difference was observed between T1 and T3 silages.

Table 2: Chemical composition of Napier grass silage treated with or without *Aspergillus* spp.

Parameter	Treatments (mean ± standard deviation)			p-value
	T1	T2	T3	
Dry matter (%)	21.0 ± 0.60^{a}	24.0 ± 0.55^{c}	23.0 ± 0.46^{b}	0.001
Crude protein (%)	9.2 ± 0.72^{a}	11.4 ± 0.51^{b}	10.2 ± 0.27^{a}	0.006
Ether extract (%)	1.5 ± 0.60^{a}	7.7 ± 1.42^{b}	0.6 ± 0.30^{a}	0.000
Crude fiber (%)	23.3 ± 0.61^{a}	29.0 ± 0.37^{c}	25.3 ± 0.56^{b}	0.000
Ash (%)	6.3 ± 0.90	9.4 ± 1.80	8.7 ± 1.37	0.083
Nitrogen-free extract (%)	59.7 ± 2.26^{b}	42.6 ± 3.02^{a}	55.5 ± 1.34^{b}	0.000

^{abc} means with different superscripts in a row differ significantly ($p<0.05$). T1 = Napier grass silage treated with 5% molasses; T2 = Napier grass silage treated with 5% molasses and *Aspergillus niger* (10 ml/kg silage; 10^6 spores/ml); T3 = Napier grass silage treated with 5% molasses and *Aspergillus awamori* (10 ml/kg silage; 10^6 spores/ml).

DISCUSSION

Fermentation characteristics

Ensiling is a process of lessening the pH value to increase the forage permanency by using an anaerobic and adequate fermentation process, as reported by Ranjit & Kung (2000). In this study, silages can be assumed to be good silage as the pH values for all treatments were in the ranges of 4.08~4.20. Hence, silages treated with *A. niger* and *A. awamori* were beneficial in balancing the pH value in Napier grass silage.

Steen et al. (1998) reported that ammonia is an essential parameter of silage DM intake. Silages that have a low content of ammonia nitrogen show that the fermentation of the silage is great. Higher values of silages with ammonia contents (>12%) for grasses signify poor fermentation. Based on the results shown in this study, the NH_3–N contents for all treatments were lower than 12%, so it can be assumed as good quality silage. Hence, the addition of *A. niger* and *A. awamori* was affected positively the ammonia nitrogen percentage of the Napier grass silage.

Lactic acid is very crucial to produce remarkably good silage. Higher content of lactic acid normally results in the lowest dry matter (DM) losses (Ward & Ondarza 2008). In this study, there were no statistical ($p>0.05$) differences in lactic acid content in Napier grass silages among the treatments. The lactic acid content in grass silage should be between 6-10% (Kung and Shaver, 2001). In this study, the range of lactic acid percentage for all treatments was between 5.75%–7.16%, which indicates that all experimental silage can be considered as good silage as the lactic acid percentages are in line with the study of Kung and Shaver (2001). The addition of *A. niger* in the silage can increase the lactic acid content, but it did not differ statistically from the control (T1). Similarly, the addition of *A. awamori* did not positively affect the lactic acid content. It indicates that *Aspergillus* sp. does not influence lactic acid production in silage. However, there is limited information on lactic acid content in Napier grass silages that are treated with *Aspergillus* sp.

Proximate components

Dry matter is an important determinant of intake and preservation values. The optimum DM for making good-quality silage should be 28-32%, as reported by Kung and Shaver (2001). However, the DM content in this study was lower than 28% for all treatments. These results might be attributed due to several factors, such as plant maturity, harvesting time, and irrigation,that may contribute to high moisture in Napier grass. The high value of DM consequently reduces the aerobic activity in the silage process. Hong et al. (2004) reported that *A. awamori* showed an increasing DM content from 88.37% to 90.94% in fermented soybean and 89.64% to 91.21% in fermented soybean meal.

Zakaria (2011) reported that the CP content of common Napier grass elevated dramatically from 8.13% to 9.26% after the ensiling process, which might be coupled with the inhabited anaerobic microorganism during the process of fermentation. The CP content of this study is similar to the findings of Oboh et al. (2002), who reported that *A. niger* has the potential for protein increment in fermented cassava flour. Similarly, Altop et al. (2018) suggested that *A. niger* could enhance the CP content in grape seed through solid-state fermentation. In another study, Li et al. (2019) noted that *A. awamori* significantly improved the CP content in soybean meals from 50.84% to 60.58%. Moreover, Webb & Wang (1997) mentioned that the addition of *A. awamori* could also improve the CP content in wheat. However, the CP content in this study using *A. awamori* was increased numerically, although there was no significant difference with the control. Hence, further investigation is needed to fully understand the mechanism of why *A. awamori* did not increase the CP content in ensiled Napier grass of this study. The CP requirements by ruminant animals to maintain their rumen environment continuation is must greater than 7%; otherwise, forage digestion will decrease (Harty & Olson, 2020) because of a lack of nitrogen for microbial growth. The silages in this study can be assumed as good silage since the CP% of ensiled Napier grass was between 9.2% to 11.4%.

It is known that the fat content in the ruminant diet should not exceed more than 7% (Palmquist, 1994); otherwise, DM intake may decrease, which can lead to reducing fiber intake and poor digestibility. In the current study, results indicate that the inclusion of *A. niger* in Napier grass silage increases the EE content. This result is parallel with the findings of Oboh et al. (2002), who observed that the fat content in cassava flour was significantly increased from 2.6% to 5.7% by the inclusion of *A. niger*. Hui et al. (2010) also reported that during fermentation, fungi produce microbial lipids in the substrate. In another study, Altop (2019) also found that the EE content was increased significantly in olive leaves by the inclusion of *A. niger*. However, the addition of *A. awamori* in Napier grass silage showed the lowest EE content, and this result is supported by Li et al. (2019), who observed that fermented soybean meal using *A. awamori* reduced the level of EE from 2.11% to 2.07%. In contrast, Hong et al. (2004) observed that fermented soybean using *A. awamori* enhanced the EE content from 18.80% to 21.62%. The mechanism of decreasing EE content in Napier grass silage using *A. awamori* is not clear. Therefore, further study is needed to improve the production efficiency of fatty acids through fungi.

The addition of *A. niger* and *A. awamori* in this study affected the CF content in the experimental silages, which is parallel with the findings of Okpako et al. (2008) and Güngör et al. (2017), who suggested that *A. niger* increased significantly in CF content in cassava peels and sour cherry kernels using solid-state fermentation. In contrast, a study by Altop (2019) concluded that the content of CF in olive leaves was decreased after fermentation with *A. niger*. Xie et al. (2016) emphasized that *A. niger* can generate cellulase in olive leaves. Hence, the decrease in CF may be due to this cellulase production. These contradictory results might be attributed to the utilization of different feed ingredients in various experiments.

Ash content in silage approximately should have less than 10%. The addition of *A. niger* and *A. awamori* in this study did not influence the content of ash in the silages. However, the study's finding is not in line with the result of Oboh et al. (2002), who reported that *A. niger* has the potential to ash increment from 2.1% to 4.5% in cassava flour. Furthermore, Altop et al. (2018) observed that *A. niger* could enhance the ash content in grape seed from 3.88% to 8.84% through solid-state fermentation. Jacobson et al. (1972) reported that the optimum calcium and phosphorus contents needed for lactating cows are 0.45% and 0.34%, respectively. Deficiency or inadequacy of mineral elements may lead to lessened feed intake and production of milk. It is important to include adequate minerals in the diet.

NFE is the major element of the rations of animal feeding stuffs, representing 40-70% of the total DM (Maynard, 1940). It helps as a source of energy for body processing and fat deposition. Fungi primarily favored soluble carbohydrates over other nutrients for carbon sources (Papagianni, 2007). The addition of *A. niger* in this study affected the NFE content in the silage. This is parallel with the study of Güngör et al. (2017), who demonstrated that the NFE content was decreased significantly in the sour cherry kernel from 38.30% to 18.05% after fermentation with *A. niger*. The decreasing mechanism in the NFE may be owing to the sugar degradation by the action of enzymes produced by *A. niger* for utilization as a source of carbon (Oboh, 2006). Carbohydrates reduced during the fermentation process may be linked to nutrient utilization by fungi for their metabolic activities and growth (Joseph et al., 2008).

CONCLUSION

Changes in fermentation characteristics and nutrient compositions of Napier grass silages are thus likely to be influenced by *Aspergillus* spp. The silage treated with *A. niger* showed higher CP and EE contents than the silage treated with *A. awamori* or without fungus. *Aspergillus niger* provided enhancement in fermentation characteristics and nutrient compositions of Napier grass silages. However, the addition of *A. awamori* did not influence the CP and EE contents of the ensiled Napier grass. The results indicated that *A. niger* could be used efficiently to make Napier grass silage. Thus, it is recommended to utilize the *A. niger* fungus since it provides a better nutritional composition in Napier grass silage than the silages treated with *A. awamori* or untreated silage.

Supplementary Materials: Not applicable

Author Contributions: M.M.R and Z.A.K: conceptualization, writing—review and editing, and writing—original draft.N.F.M.S, M.L.N, N.U, N.D.R, K.M, G.T.I, A.B.T, L.S.W: Writing – review and editing, conceptualization and data analysis.

Funding: This research was funded by USDA-NIFA Sustainable Agriculture Systems, Grant No. 2019-69012-29905. Title of Project: Empowering US Broiler Production for Transformation and Sustainability USDA-NIFA (Sustainable Agriculture Systems): No. 2019-69012-29905.

Data Availability Statement: The data that support the findings of this study are available on request from the corresponding author

Acknowledgments: The research article is from the collaboration between Universiti Malaysia Kelantan and University of Arkansas. These collaborations are a part of the planning by Advanced Livestock and Aquaculture Research Group – ALAReG under the Faculty of Agro-Based Industry, Universiti Malaysia Kelantan, Jeli Campus.

Conflicts of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

REFERENCES

1. Altop, A. (2019). Effect of solid-state fermentation on main nutritional components, some minerals, condensed tannin and phenolic compounds of olive leaves. Turkish Journal of Agriculture - Food Science and Technology 7(1): 115. https://doi.org/10.24925/turjaf.v7i1.115-119.2231
2. Altop, A., Güngör & Erener G. (2018). Aspergillus niger may improve nutritional quality of grape seed and its usability in animal nutrition through solid-state fermentation. International Advanced Researches and Engineering Journal 2(3): 273–277.
3. Amin AM, Jaafar Z, Khim NL. (2004). Effect of salt on tempoyak fermentation and sensory evaluation. Journal Biological Sciences 4(5): 650–653.

4. AOAC (Association of Official Analytical Chemists). (2000). Official methods of analysis of AOAC. International 17th edition; Gaithersburg, MD, USA Association of Analytical Communities, 2200 pp.
5. De Morais, R. F., de Souza, B. J., Leite, J. M., de Barros Soares, L. H., Rodrigues Alves, B. J., Boddey, R. M., & Urquiaga, S. (2009). Genótipos de capim-elefante para produção de bioenergia por combustão direta da biomassa. Pesquisa Agropecuaria Brasileira 44(2): 133–140. https://doi.org/10.1590/S0100-204X2009000200004
6. Güngör, E., Altop, A., Öztürk, E., & Erener, G. (2017). Nutritional Changes of sour cherry (Prunus cerasus) kernel subjected to Aspergillus niger solid-state fermentation. The Special Issue of 2nd International Balkan Agriculture Congress, Journal of Tekirdag Agricultural Faculty, pp. 99–103.
7. Gwayumba, W., Christensen, D. A., McKinnon, J. J., & Yu, P. (2002). Dry matter intake, digestibility and milk yield by Friesian cows fed two Napier grass varieties. Asian Australasian Journal of Animal Sciences 15: 516–521.
8. Harty, A., & Olson, K. (2020). Nutrient Requirements of Beef Cows. Chapter 14, SDSU Extension, South Dakota Board of Regents. Retrieved on 22 November 2022 from https://extension.sdstate.edu/sites/default/files/2021-05/S-0013-14.pdf
9. Hong, K.-J., Kim, S. W., & Lee, C.-H. (2004). Aspergillus oryzae GB-107 fermentation improves nutritional quality of food soybeans and feed soybean meals. Journal of Medicinal Food 7(4): 430–435. https://doi.org/10.1089/jmf.2004.7.430
10. Hui, L., Wan, C., Hai-Tao, D., Xue-Jiao, C., Qi-Fa, Z., & Yu-Hua, Z. (2010). Direct microbial conversion of wheat straw into lipid by a cellulolytic fungus of Aspergillus oryzae A-4 in solid-state fermentation. Bioresource Technology 101(19): 7556–7562. https://doi.org/ 10.1016/ J.BIORTECH.2010.04.027
11. Jacobson, D. R., Hemken, R. W., Button, F. S., & Hatton, R. H. (1972). Mineral nutrition, calcium, phosphorus, magnesium, and potassium interrelationships. Journal of Dairy Science 55(7): 935–944. https://doi.org/10.3168/ JDS. S0022-0302(72)85600-5
12. Joseph I, Raj RP, Bhatnagar D. 2008. Effect of solid-state fermentation on nutrient composition of selected feed ingredients. Indian Journal of Fisheries 55: 327-332.
13. Kung L and Shaver R. 2001. Interpretation and use of silage fermentation analysis reports. Focus on Forage 3(13): 1–5.
14. Lee, C. N., Fukumoto, G. K., Thorn, M. S., Stevenson, M. H., Nakahata, M., & Ogoshi, R. M. (2016). Bana grass (Pennisetum purpureum): A possible forage for ruminants in Hawai. Pasture and Range Management, PRM-11 (Issue July). Retrieved on 22 November 2022 from https://www.ctahr.hawaii.edu/oc/freepubs/pdf/PRM-11.pdf
15. Li, C., Zhang, B., Zhou, H., Wang, X., Pi, X., Wang, X., Mai, K., & He, G. (2019). Beneficial influences of dietary Aspergillus awamori fermented soybean meal on oxidative homoeostasis and inflammatory response in turbot (Scophthalmus maximus L.). Fish and Shellfish Immunology 93: 8–16. https://doi.org/10.1016/J.FSI.2019.07.037
16. Madamwar, D., Patel, S., & Parikh, H. (1989). Solid state fermentation for cellulases and β-glucosidase production by Aspergillus niger. Journal of Fermentation and Bioengineering 67(6): 424–426. https://doi.org/10.1016/0922-338X(89)90150-5
17. Magcale-Macandog, D. B., Predo, C. D., Menz, K. M., & Calub, A. D. (1998). Napier grass strips and livestock: a bioeconomic analysis. Agroforestry Systems 40(1): 41–58. https://doi.org/ 10.1023/A:1006065626800
18. Maynard, L. A. (1940). Nitrogen-free extract in animal nutrition. Journal of AOAC INTERNATIONAL 23(1): 156–161. https://doi.org/10.1093/JAOAC/23.1.156
19. Morgado, H. S., Cysneiros, C. S. S., Sousa, C. M., Stringhini, J. H., Ulhoa, C. J., Silva, A. S., Fabino Neto, R. v.,

Freitas, P. V. D. X., Oliveira, H. P., & Batista, L. H. C. (2016). Addition of amylase from Aspergillus awamori to the diet of broiler chickens. Revista Brasileira de Ciencia Avicola 18(4): 725–732. https://doi.org/10.1590/1516-635x1804725-732

20. Naher L, Tan SG, Yusuf UK, Ho CL, Abdullah F. (2012). Biocontrol agent Trichoderma harzianum strain FA 1132 as an enhancer of oil palm growth. Pertanika Journal of Tropical Agricultural Science 35: 173–182.
21. Oboh, G. (2006). Nutrient enrichment of cassava peels using a mixed culture of Saccharomyces cerevisae and Lactobacillus spp solid media fermentation techniques. Electronic Journal of Biotechnology 9(1): 46–49. https://doi.org/10.2225/VOL9-ISSUE1-FULLTEXT-1
22. Oboh, G., Akindahunsi, A. A., & Oshodi, A. A. (2002). Nutrient and anti-nutrient contents of Aspergillus niger -fermented cassava products (flour and gari). Journal of Food Composition and Analysis 15(5): 617–622. https://doi.org/10.1006/jfca.2002.1065
23. Okpako, C. E., Ntui, V. O., Osuagwu, A. N., & Obasi, F. I. (2008). Proximate composition and cyanide content of cassava peels fermented with Aspergillus niger and Lactobacillus rhamnosus. Journal of Food, Agriculture and Environment 6(2): 251–255.
24. Oriol, E., Raimbault, M., Roussos, S., & Viniegra-Gonzales, G. (1988). Water and water activity in the solid-state fermentation of cassava starch by Aspergillus niger. Applied Microbiology and Biotechnology 27(5–6): 498–503. https://doi.org/10.1007/BF00451620
25. Oyeleke S. B., Oyewole O. A., & Egwim E. C. (2012). Production of protease and amylase from Bacillus subtilis and Aspergillus niger using Parkia biglobossa (Africa Locust Beans) as substrate in solid state fermentation. Advances in Life Sciences 1(2): 49–53. https://doi.org/10.5923/j.als.20110102.09
26. Palmquist, D. L. (1994). The role of dietary fats in efficiency of ruminants. Journal of Nutrition 124(8 SUPPL.). https://doi.org/10.1093/JN/124.SUPPL_8.1377S
27. Papagianni, M. (2007). Advances in citric acid fermentation by Aspergillus niger: biochemical aspects, membrane transport and modeling. Biotechnology Advances 25(3): 244–263. https://doi.org/10.1016/J.BIOTECHADV.2007.01.002
28. Pitt, J. I., & Hocking, A. D. (2009). Fungi and food spoilage. Springer New York, NY. https://doi.org/10.1007/978-0-387-92207-2
29. Ranjit, N. K., & Kung, L. (2000). The effect of Lactobacillus buchneri, Lactobacillus plantarum, or a chemical preservative on the fermentation and aerobic stability of corn silage. Journal of Dairy Science 83(3): 526–535. https://doi.org/10.3168/jds.S0022-0302(00)74912-5
30. Steen, R. W. J., Gordon, F. J., Dawson, L. E. R., Park, R. S., Mayne, C. S., Agnew, R. E., Kilpatrick, D. J., & Porter, M. G. (1998). Factors affecting the intake of grass silage by cattle and prediction of silage intake. Animal Science 66(1): 115–127. https://doi.org/10. 1017/S1357729800008894
31. Tsai, W. T., & Tsai, Y. L. (2016). Thermochemical characterization of Napier grass as an energy source and its environmental and economic benefit analysis. Energy Sources, Part B: Economics, Planning and Policy 11(2): 130–136. https://doi.org/10.1080/ 15567249. 2011.590847
32. Van den Berg, J., & Van Hamburg, H. (2015). Trap cropping with Napier grass, Pennisetum purpureum (Schumach), decreases damage by maize stem borers. International Journal of Pest Management 61(1): 73–79. https://doi.org/10.1080/09670874.2014.999733
33. Ward, R. T., & Ondarza, M. B. de. (2008). Fermentation analysis of silage: use and interpretation (Issue 301). Retrieved on 22 November 2022 from ttps://www.foragelab.com/Media/Fermentation-Silage-NFMP-Oct-2008.pdf

34. Webb, C., Wang, R. (1997). Development of a Generic Fermentation Feedstock from Whole Wheat Flour. In: Campbell, G.M., Webb, C., McKee, S.L. (eds) Cereals. Springer, Boston, MA. https://doi.org/10.1007/978-1-4757-2675-6_25
35. Xie, P. J., Huang, L. X., Zhang, C. H., & Zhang, Y. L. (2016). Nutrient assessment of olive leaf residues processed by solid-state fermentation as an innovative feedstuff additive. Journal of Applied Microbiology 121(1): 28–40. https://doi.org/10.1111/JAM.13131
36. Zakaria, A. (2011). Characterization and potential use of lactic acid bacteria isolated from corn silage. Masters thesis, Universiti Putra Malaysia. Retrieved on 22 November 2022 from http://psasir.upm.edu.my/id/eprint/25912/