Effect of *Gliricidia sepium* leaves and molasses inclusion on aerobic stability, value and digestibility of Napier grass silage

Jusoh, S.*, Alimon, A.R. and Iman, M.N.
Department of Animal Science, Faculty of Agriculture,
Universiti Putra Malaysia, Serdang, Selangor
*Corresponding author: shokri@upm.edu.my

Abstract

Most tropical roughage feeds are generally deficient in nitrogen, energy or some minerals and vitamins. A study was conducted to evaluate the effect of adding Gliricidia (*Gliricidia sepium)* leaves and molasses to Napier grass (*Pennisetum purpureum*) silage. Silage were prepared from Napier grass and mixed with Gliricidia and molasses. There were four treatments involved which were NG, containing Napier grass only which was the experiment control, GS containing Napier grass mixed with 5% Gliricidia leaves, ML containing the Napier grass mixed with 5% molasses, and MG containing Napier grass mixed with 5% molasses and 5% Gliricidia leaves. The treatments were analyzed for aerobic stability, dry matter (DM), crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL), and digestibility. There were no significant differences in aerobic stability of silage between treatments, and the treatments with Gliricidia were higher in crude protein ($p<0.05$) and had better digestibility ($p<0.05$) when compared to the control. In conclusion, Gliricidia leaves as additives to Napier grass silage can improve its nutritive value and quality.

Keywords: grass silage, Napier grass, aerobic stability, nutritive value

Introduction

Most tropical roughage based feeds are generally deficient in protein, energy or some minerals and vitamins resulting in they become less adequate to support animal production. The mostly used forage species in Malaysia is Napier grass (*Pennisetum pupureum*) in cut and carry system, either in the form of fresh grass or silage as source of feeds.

Napier grass is widely planted and sometimes conserved as silage. However, it has high moisture content and insufficient quantity of water soluble carbohydrates at vegetative stage, which sometimes resulted in clostridial fermentation (Yahaya *et al*., 2004). Furthermore, its low crude protein content (Yunus, Ohba and Shimojo, 2000) and high structural carbohydrate contents (Zhang *et al*., 2011; Bureenok *et al*., 2012) usually lead to low nutritive value of silage. Molasses, the byproduct of sugar industry, is often used for ensiling low water soluble carbohydrate forages, such as legumes and tropical grasses, and are widely used and combined successfully with multiple ingredients to alter its nutritional profile. Several workers have concluded that the addition of molasses increased the dry matter and lactic acid contents and reduced the pH and ammonia nitrogen content in treated silages (Tjandraatmadja *et al*., 1994; Bilal, 2009).

Supplementing Napier grass fodder with foliage from tree legumes, such as *Leucaena leucocephala* and *Gliricidia sepium*, could improve the dry matter intake and live weight gain of young cattle and lactation performance of dairy cows. Gliricidia species is now widely planted in smallholder farming systems to enhance soil fertility through nitrogen fixation and green manure and to provide high quality fodder for livestock, particularly dairy cows in smallholder farming systems, and used as live fence, shade, windbreak, boundary marking, firewood and ornamental. Inclusion of legume leaves in silage is believed to increase the quality of silage and improve the feeding value of *Gliricidia sepium* leaves in terms of aerobic stability. Its chemical composition shows a highly nutritious fodder, high in protein, low in fibre and highly digestible. Therefore, it can be used to enhance the quality of tropical forages in terms of nutritive value and digestibility.

The inclusion of molasses and Gliricidia leaves in Napier grass silage would increase the quality and nutritive value of the silage, and by increasing the quality of silage, it can supply good quality feed to the animals, thus decreasing the need of purchased concentrate which reduces the feeding cost of animals. Therefore, the objectives of this study were to evaluate the effect of adding legume leaves and molasses on aerobic stability of silage and to determine the nutrient contents of Napier grass silage mixed with legume leaves and molasses.

Materials and Methods

This experiment was conducted at Field 2, Universiti Putra Malaysia (UPM), Serdang Selangor from November 2013 until January 2014. The proximate analysis was conducted at Nutrition Laboratory and *in vitro* digestibility test was conducted at Pasture Laboratory, Department of Animal Science, Faculty of Agriculture, UPM from February to March 2014. The cultivar of Napier grass was Taiwan Napier, managed with normal agronomic practices such as fertilization with 60 kg N, 30 kg P and 30 kg K at establishment stage. The soil type of the area is clay as classified by Soil Taxonomy Classification (USDA).

Treatments and silage making process

Napier grass was harvested and cut 20 cm above the ground level at 4, 6 and 8 wk harvesting age. The cutting was done manually and the grass was left to wilt in the field for 5 h. Later, the grass was chopped into 1-2 cm chops using a mechanical chopper. The chopped grass were later placed in plastic 25-kg drums and compacted by foot. The drums were covered with lids and clamped to keep the anaerobic condition during storage. Approximately 25 kg of the chopped grass were mixed with the respective treatments, and during sampling samples were divided into two portions for fresh chemical composition and dried in 65°C oven for laboratory analyses.

The experiment consisted of four treatments with three replicates for each treatment, using Napier grass age of 10 wk and ensiled as silage with Napier grass only (NG) as control, mixed with 5% *Gliricidia sepium* (GS), with 5% molasses (ML), and with 5% molasses and 5% *Gliricidia sepium* (MG) on fresh weight basis. The materials were compacted in plastic drums with average weight of 25 kg each. Each replicate was sampled and analysed for its aerobic stability, nutritive value and *in vitro* digestibility.

Silage samples were taken for analyses after 4 wk of fermentation, from each replicate at the top, mid and bottom part of the drum and were mixed thoroughly before taking subsamples. Approximately 200 g of the samples from each treatment were placed in paper bags and dried in the oven at 60°C for 72 h. The samples were ground to pass 1-mm sieve and kept for further analyses. A portion of sample from each treatment was subjected to aerobic stability test.

Aerobic stability test

The aerobic stability test was conducted by measuring the pH changes and mold formation on the silage after being exposed to aerobic condition for 5 d. About 10 g of silage samples taken from each treatment, were mixed with 50 mL of distilled water, and pH was measured using a pH meter (Extech pH Meter). The measurement of pH was taken again after 5 d of aerobic exposure. The mold formation of the silage was also observed after 5 d of aerobic exposure. Visual appearance of the samples after being exposed to the air was performed by looking at the extent of mold cover, texture and odour. The evaluation was converted into a numeric scale from 1 to 5, with 1 being good quality silage with no apparent molding and 5 being completely molded samples.

Chemical analysis

The proximate analysis of dried samples was analyzed for crude protein (CP), neutral detergent fibre (NDF), acid detergent fibre (ADF), acid detergent lignin (ADL) according to standard procedure (AOAC, 1984).

In-vitro gas production analysis

Rumen fluid was collected from donor goats located at Field 2, UPM, and dried sample was incubated with a mixture of rumen fluid and buffer solution for 48 h. The readings were recorded at 4, 8, 12, 24 and 32 h. Gas production that indicated digestibility was measured. The silage digestibility was evaluated using *in-vitro* gas production technique.

Statistical analysis

A two-way analysis of variance (ANOVA) was carried for the experimental data using the Statistical Analysis System software (SAS, 2011) with DMRT was used to indicate differences in means of treatments.at $p<0.05$ to indicate the level of significance.

Results and Discussion

The main goal of silage making is to maintain the original quality of the preserved crop as much as possible. To achieve this, additives and inoculants have been used for several decades to direct the fermentation process towards the production of lactic acid as the main fermentation product. Dunière *et al*. (2013) reviewed that to improve silage preservation and guarantee the quality of silage as animal feed, silage additives such as chemicals, enzymes and bacterial agents can be employed.

Silage aerobic stability

There was an increase in pH of silage samples from 3.1 at day 1 to 6.2 at day 5 across all treatments indicating the breakdown of lactic acid, making it to be exposed to deterioration process. There were no significant differences in pH changes between treatments, and it showed that the pH changes between treatments were not significantly different after 5 d of aerobic exposure, thus indicating there was no significant effect of legume leaves or molasses inclusion on the aerobic stability of Napier grass silage (Figure 1). The mold formation test showed that there was no significant difference ($p>0.05$) between treatments, where the silage with the mixture of molasses and Gliricidia (MG) showed more mold formation compared to the other treatments (Figure 2). This result showed that the inclusion of legumes and molasses in grass silage did not increase the aerobic stability of the silage.

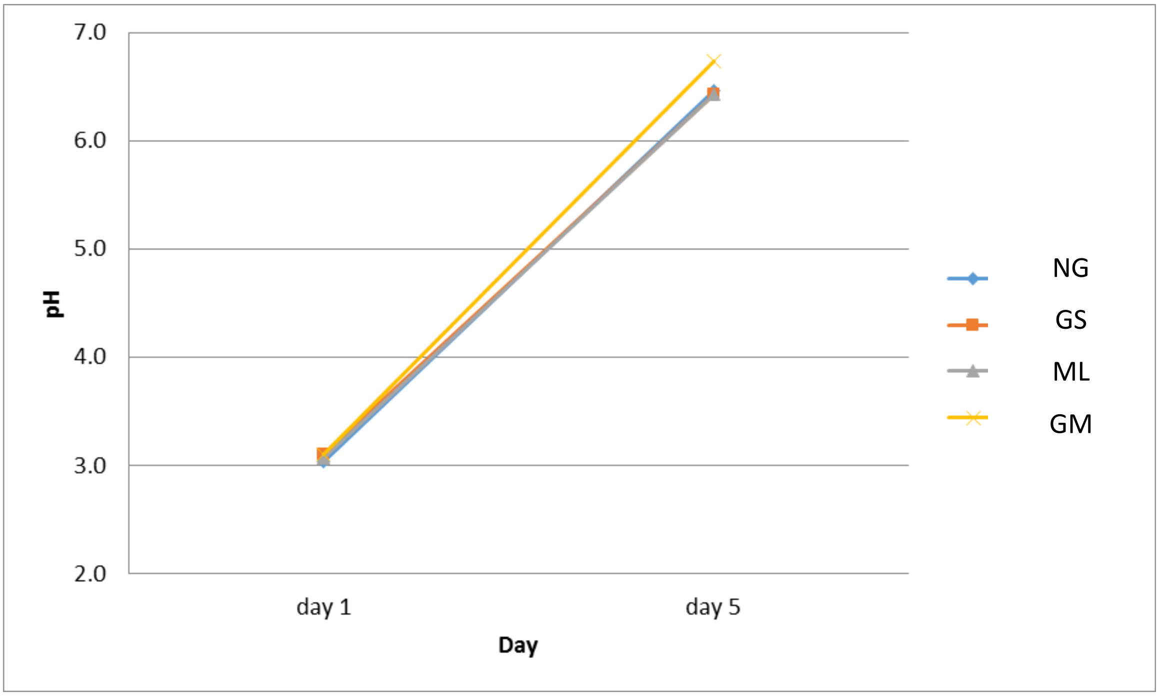

Figure 1. The pH changes of silage samples after 5 days of exposure to aerobic condition

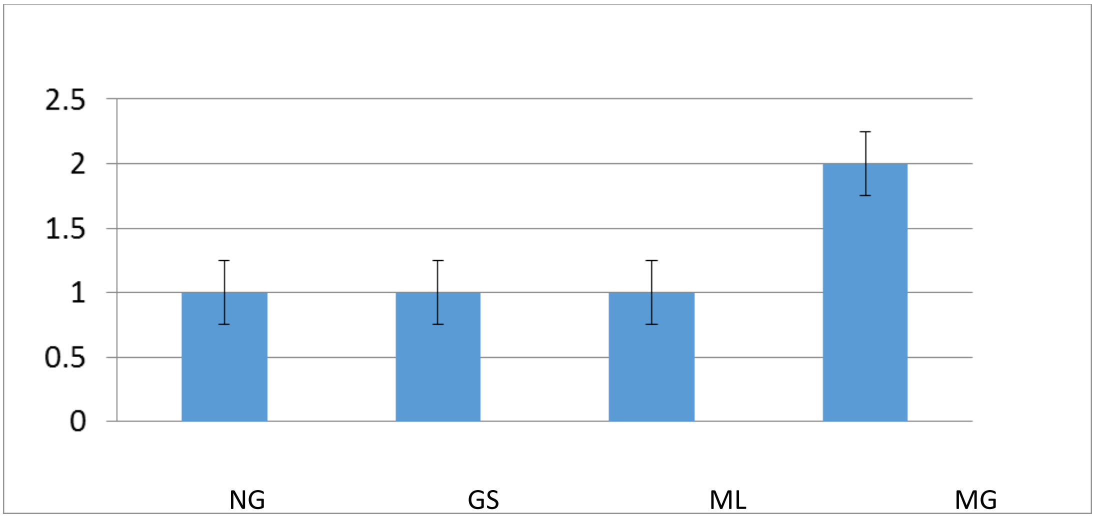

Figure 2. Mold formation of each treatment after 5 days of exposure to aerobic condition

The pH of silage is a key criterion to evaluate quality, with generally the lower the pH, the better preserved and more stable the silage. In the present study, the silage pH across the treatments was around 3.0, indicating that good quality silage had been produced regardless of type of additives used. However, the aerobic stability test showed that the inclusion of legumes and molasses in grass silage did not influence the pH changes after aerobic exposure indicating that there was no difference in aerobic stability of the silage.

Johnson *et al*. (1999) reported that inoculation of corn silage with lactic acid producing bacteria (LAB) tended to improve aerobic stability of corn silage more than maturity and mechanical processing. The inoculation of homofermentative LAB successfully reduced pH of both precision-chopped and long cut silages but abundant production of lactic acid did not secure the aerobic stability of these silages (Knický, 2005) The result of this study showed that there were no significant effects of additives in aerobic stability of the silage, as the silage pH increased after exposure to air, and there was no significant difference between the treatments. This is because yeasts degrade lactic acid in the presence of air and their primary microbes cause increase in pH. Therefore, the formation of mold also is one of the parameters of silage quality. The result showed that the GS, ML, and MG treatments had significant differences in mold formation, whereas NG had no significant difference. Acetic acid is known as an inhibitor of yeast and mold growth (Moon, 1983). However, it appears that silage pH is a factor that affects voluntary consumption of corn silage and that pH 5 to 6 is optimum, whereas silage pH above and below optimum may reduce intake (Shaver *et al*., 1984). However, wilting tends to reduce acetic acid and ammonia-N contents and improve the fermentation quality of legume silage. The wilted silage or silage stored at low temperature had poor aerobic stability (Liu *et al*., 2011).

Chemical analysis

The chemical composition of the silage sample showed that the crude protein was higher ($p<0.05$) in GS (6.94%) and MG (6.69%) compared to NG (5.53%) and ML (5.46%). The neutral detergent fibre (NDF) content of NG was the highest with 76.05% NDF, compared to GS which was 74.10%, ML 71.56%, and MG 70.78%, showing significant differences ($p<0.05$) between GS, ML, and MG, when compared to NG (control).

The ADF content of NG was significantly the highest (52.35%) compared to GS (45.25%), ML (40.82%) and MG (42.61%). MG was contained significantly highest percentage of ADL (56.52%), followed by GS (53.83%), NG (44.59%) and ML (36.81%) was the lowest. There was significant difference between GS and MG when compared to NG, and there was no significant difference with ML (Table 1).

Table 1 Chemical composition of silage treated with *Gliricidia sepium* leaves and molasses

Composition (%)	NG	GS	ML	MG
CP	5.529 ± 0.172[a]	5.461 ± 0.078[a]	6.944 ± 0.078[b]	6.693 ± 0.358[b]
NDF	76.932 ± 0.505[a]	71.560±0.121[b]	74.094±1.047[b]	70.784±0.875[b]
ADF	52.352 ± 0.806[a]	40.821±0.666[b]	45.247±1.224[b]	42.607±0.656[b]
ADL	44.590 ± 5.428[a]	36.815 ± 2.015[a]	53.825±0.579[b]	56.524±1.828[b]

* Significance different at $p<0.001$

The results showed that GS and MG which contained Gliricidia had the highest crude protein content, with 6.94% and 6.69%, respectively. Legumes have higher protein content than grasses and can be a great benefit to help meet livestock's protein intake need. This showed that by adding Gliricidia, it could increase the protein content of silage thus increasing its nutritive value. This was in line with Phiri *et al*. (2007) who reported that the ammonia-N content was highest in legume maize silage and lowest in maize silage. Dewhurst (2013) outlined a further benefit of legumes and maize of reduced rate of decline in digestibility. Legume silages often lead to a reduction in milk fat concentration and increased levels of polyunsaturated fatty acids, 18:2 n-6 and 18:3 n-3. This latter effect is related to reduce rumen biohydrogenation as a consequence of increased rumen passage rates or the effects of polyphenol oxidase.

Neutral detergent fibre (NDF) consists of the total fibre content which contains cellulose, hemicellulose, and lignin. The result showed that there was significant reduction in NDF when silage was added with legume leaves or molasses, indicating an improvement in feed quality. This was in agreement with Ajayi (2011) that the digestibility of the fibre fractions was highest in lima bean/grass silage followed by pigeon pea/grass silage, and the least percent digestibility was observed in goats fed elephant grass silage. The non-soluble material is categorized as a neutral detergent fibre as the plant grows the NDF will increase due to a rise in degradable fraction of NDF. Traxler *et al*. (1998) reported that neutral detergent fibre indigestibility increased nonlinearly as the lignin concentration of the NDF increased, and the legume forage with a low NDF content was usually of high digestibility and species with high lignin content were often of low digestibility. As NDF values increase total feed intake will decrease. Legumes contain low NDF than grasses at a comparable stage of maturity and in this experiment, there were significant differences in GS, ML, and MG. MG which contained Gliricidia and molasses have the lowest NDF content. Thus, the lower NDF value indicated increase in total feed intake and MG had the highest ability to increase feed intake in ruminants. Armentano and Pereira (1997) measured effectiveness of a high fibre feed which differred when estimated by chewing, ratio of acetate to propionate, or milk fat concentration. In all cases, inclusion of negative control treatments was necessary to measure the effect of removing fibre without introducing a substitute fibre source.

There were significant differences in ADF content in ML and MG when compared to the control and no significant difference in GS, as ADF increases and the digestibility of a forage usually decreases because of the increase in lignin content. However, in ADF, only cellulose and lignin pyrolysis peaks were detected and a hemicellulose decomposition peak or shoulder was not present (Sharma, 1996).

With advancing growth and maturity, forage cells insert a non-carbohydrate material, known as lignin, into the primary and secondary walls. Lignin can be thought of as the primary skeleton of the plant cell. It is important from a nutritional perspective because it is a non-digestible substance and its presence will inhibit the availability of the cellulose and hemicellulose portions of the forage (Meier, Kreuzer and Marquardt, 2012). Lignin is a polymer component of the cell walls that provides rigidity and structural support to plants and cannot be digested by animal enzymes. It increases as a plant matures and is higher for the same plant species grown under warm weather conditions. Among the fibre fractions which are hemicelluloses, cellulose, and lignin, the cellulose is the major component that can be digested by animals. However, lignin can bind up the cellulose fraction and lower the digestibility. The higher the concentration of lignin the less digestible the fibre will be. The lignocellulosic biomass represents a rather unused source for biogas and ethanol production. Many factors, like lignin content, crystallinity of cellulose, and particle size, limit the digestibility of the hemicellulose and cellulose present in the lignocellulosic biomass (Hendriks and Zeeman, 2009). From the result, it showed a significant difference of ADL in ML and GM when compared to NG, and no significant difference between NG and GS.

In –vitro as production digestibility

GS and MG had the highest digestibility compared to the NG and ML. There were a significant difference in GS and MG and there was no significant difference in ML (Figure 3). GS and MG which contained Gliricidia and molasses showed significantly higher digestibility when compared to NG (control). ML containing only molasses showed no significant difference. The study showed that degradation of the neutral detergent-soluble fraction in grasses and legumes found the presence of fast digesting material in legumes. Hackmann *et al.* (2008) revealed that digestible DMI and degradation parameter estimates were related for grass but not for alfalfa forages. The feeding value of Gliricidia leaves in terms of chemical composition showed a highly nutritious fodder - high in protein, low in fibre and was highly digestible. Thus, according to previous research, it is proven that Gliricidia can improve the digestibility of tropical forages. Supplementing poor quality basal grass diets with legume forage has been shown to increase feed intake and diet digestibility by ruminant livestock (Minson and Milford, 1967).

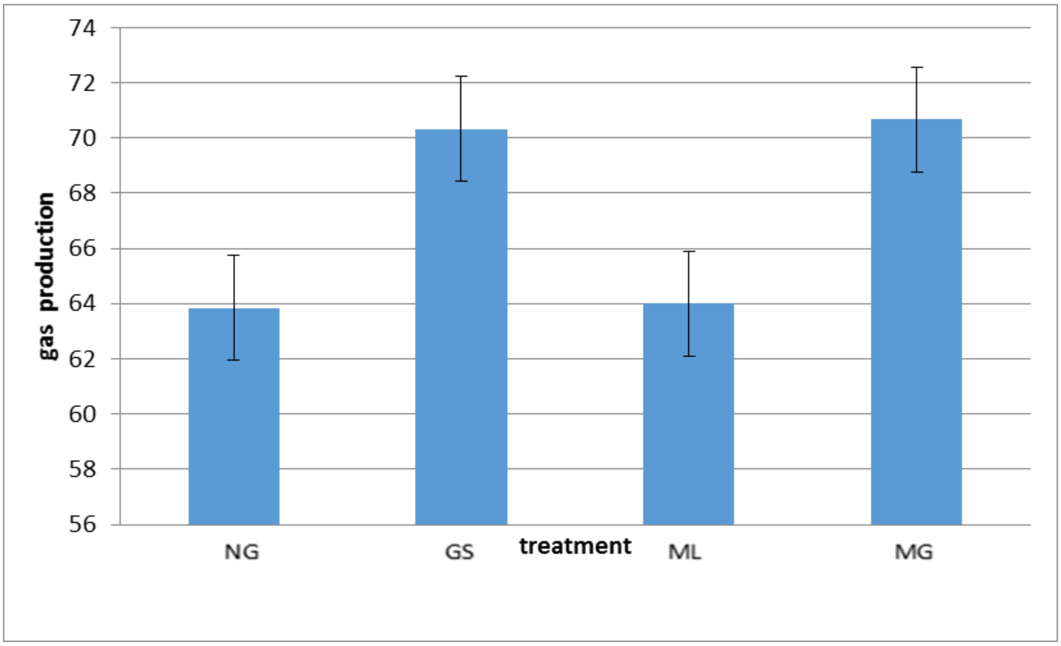

Figure 3. The total gas production of silage samples

Conclusion

Gliricidia sepium (Gliricidia) leaves and molasses as additives have very good potential to increase the quality and nutritive value of Napier grass silage. The quality parameters that have been measured indicate that by adding Gliricidia leaves can increase the crude protein content and digestibility of silage. Adding molasses also can improve nutritive value of Napier grass silage. It is concluded that adding molasses and legume leaves such as *Gliricidia sepium* leaves can increase the quality of the low quality grass silage with increases in nutritive value and digestibility. Therefore, adding molasses and *Gliricidia sepium* can be practiced by the farmers to enhance the nutritive value of feed based on Napier grass silage.

References

Ajayi, F.T. 2011. Effects of feeding ensiled mixtures of elephant grass (Pennisetum purpureum) with three grain legume plants on digestibility and nitrogen balance of West African Dwarf goats. *Livestock Sci*., 142: 80–84. doi:10.1016/j.livsci.2011.06.020

Armentano, L, and Pereira, M. 1997. Measuring the effectiveness of fiber by animal response trials. *J. Dairy Sci*. 80: 1416–1425. doi:10.3168/jds.S0022-0302(97)76071-5

Bureenok, S., Yuangklang, C., Vasupen, K., Schonewille, J. and Kawamoto, Y. 2012. The effects of additives in Napier grass silages on chemical composition, feed intake, nutrient digestibility and rumen fermentation. *Asian-Aust J Anim Sci*. 25: 1248–1254.

Dewhurst, R.J. 2013. Milk production from silage: Comparison of grass, legume and maize silages and their mixtures. *Agricultural and Food Science*. Retrieved from http://www.scopus.com/inward/record.url?eid=2-s2.0-84878698655&partnerID=tZOtx3y1

Dunière, L., Sindou, J., Chaucheyras-Durand, F., Chevallier, I. and Thévenot-Sergentet, D. 2013. Silage processing and strategies to prevent persistence of undesirable microorganisms. *Anim. Feed Sci. and Tech.* doi:10.1016/j.anifeedsci.2013.04.006

Hackmann, T.J., Sampson, J.D., and Spain, J.N. 2008. Comparing relative feed value with degradation parameters of grass and legume forages. *J. Anim.Sci*. 86: 2344–2356. doi:10.2527/jas.2007-0545

Hendriks, A. and Zeeman, G. 2009. Pretreatments to enhance the digestibility of lignocellulosic biomass. *Bioresource Tech*. doi:10.1016/j.biortech.2008.05.027

Johnson, L., Harrison, J.H., Hunt, C., Shinners, K., Doggett, C.G. and Sapienza, D. 1999. Nutritive value of corn silage as affected by maturity and mechanical processing: a contemporary review. *J. Dairy Sci.* 82: 2813–2825. doi:10.3168/jds.S0022-0302(99)75540-2

Knický, M. 2005. Possibilities to Improve Silage Conservation: Effects of Crop, Ensiling Technology and Additives. Acta Universitatis Agriculturae Sueciae, ISSN 1652-6880

Yunus, M., Ohba, N., Shimojo, M,. Furuse, M. and Masuda, Y. 2000. Effects of adding urea and molasses on Napier grass silage quality. *Asian-Aus. J. Anim. Sci.* 13(11): 1542 – 1547.

Phiri, M.S., Ngongoni, N.T., Maasdorp, B.V., Titterton, M., Mupangwa, J.F. and Sebata, A. 2007. Ensiling characteristics and feeding value of silage made from browse tree legume-maize mixtures. *Tropical and Subtropical Agroecosystems*, 7: 149–156.

Meier, J.S., Kreuzer, M. and Marquardt, S. 2012. Design and methodology of choice feeding experiments with ruminant livestock. *Applied Animal Behaviour Sci*. doi:10.1016/j.applanim.2012.04.008

Minson, D.J. and Milford, R. 1967. The voluntary intake and digestibility of diets containing different proportions of legume and mature Pangola grass (*Digitaria decumbens*). *Australian J. Experimental Agric. and Animal Husbandry*, 7: 745.

Moon, N. 1983. Inhibition of the growth of acid tolerant yeasts by acetate, lactate and propionate and their synergistic mixtures. *J.Applied Bacteriology*, 55: 454–460.

Bilal, M.Q. 2009. Effect of molasses and corn as silage additives on the characteristics of Mott Dwarf elephant grass silage at different fermentation periods. *Pak Vet J.* 29: 19–23.

SAS. 2011. SAS/STAT 9.3 User’s Guide. User’s Guide. SAS Institute Inc., Cary, NC. Retrieved from http://scholar.google.com/scholar?hl=en&btnG=Search&q=intitle:Sas/stat+9.3#1

Sharma, H.S.S. 1996. Compositional analysis of neutral detergent, acid detergent, lignin and humus fractions of mushroom compost. *Thermochimica Acta*, 285: 211–220. doi:10.1016/0040-6031(96)02954-1

Tjandraatmadja, M., Norton, B. and MacRae, I. 1994. Ensilage characteristics of three tropical grasses as influenced by stage of growth and addition of molasses. *World J.Microbiol Biotechnol*. 10: 74–81.

Yahaya, M.S., Goto, M., Yimiti, W., Smerjai, B. and Kawamoto, Y. 2004. Evaluation of fermentation quality of a tropical and temperate forage crops ensiled with additives of fermented juice of epiphytic lactic acid bacteria (FJLB). *Asian-Aust J Anim Sci*. 17: 942–946.

Zhang, L., Yu, C.Q., Shimojo, M. and Shao, T. 2011. Effect of different rates of ethanol additive on fermentation quality of Napier grass (*Pennisetum purpureum*). *Asian-Aust J Anim Sci*. 24: 636–642.