J. Agrobiotech. **Vol 6**, 2015, p. X-XX.

ISSN 1985-5133 (Press)
ISSN 2180-1983 (Online)

Short Communication

Comparative Composition of Napier Silage Prepared by Using Conventional and Probiotic Methods

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ABSTRACT

This study was conducted to evaluate the efficiency of probiotics to improve its nutrient contents when Napier (*Pennisetum purpureum*) is ensiled this way, compared to using the conventional method. Napier grass was chopped and one portion was ensiled anaerobically by compacting it into the conventional blue plastic drum, while the other portion of a half tonne was prepared by spraying and mixing with three types of probiotics, the BIOBAC 1, 2 and 3, made up of bacteria, fungus and enzymes. The probiotics were diluted with water at the rate of 100 mL per tonne for each type, and placed into a haversack sprayer and sprayed and mixed into the material. They were then heaped into the shape of a mount in a concrete floored housing and left to ferment and mature for 21 days. Only during the first five days did the heap need to be moist, to allow for the fungus to somewhat germinate, by spraying with just plain water over the top of the mount if necessary. After that it was left as it was to mature and dry. The conventional silage was prepared by using the anaerobic fermentation method in the traditional plastic drum and was also left to mature over 21 days. After the 21-day period, ensiling samples from both types of silage were taken, dried and subjected to chemical evaluation in the laboratory using the methods recommended by the Association of Official Analytical Chemist (AOAC) for comparison. The proximate analysis of both silages was conducted at the Malaysian Veterinary Institute, Kluang, Johor. In the probiotic silage, percentage of nutrients dry matter content (DM), crude protein (CP), crude fat (CF), crude fiber (CF), ash, Calcium, Phosphorus, Nitrogen free extract (NFE), total digestible nutrients (TDN), metabolizable energy (ME) and pH were determined at 65.06%, 9.50%, 0.84%, 42.72%, 6.92%, 0.18%, 0.16%, 40.06%, 49.48%, 7.25 MJ/Kg dan 6.82%, respectively. Whilst for the conventional silage the nutrient contents were determined at 23.40%, 6.26%, 1.92%, 49.56%, 3.96%, 0.08%, 0.09%, 38.26%, 42.22%, 6.06 MJ/Kg dan 5.51%, respectively. The nutrient contents of OPF silage prepared by the probiotic method were all significantly higher than those of conventional OPF silage. This study shows that the probiotics method has highly significantly improved some of the nutrient contents of Napier silage, although the EE, CF, ash and pH showed non-significance, which indicated that the probiotics method could not improve the contents of all nutrients in silage. Thus, probiotics is a potential alternative silage making tool in Malaysia which would result in value-added properties in the resulting silage, especially for protein and energy, the most important nutrients needed in animal feeds.

Keywords: Conventional silage, probiotics, fermentation, proximate analysis

ABSTRAK

Kajian ini bertujuan untuk menilai kecekapan probiotik dalam memperbaiki kandungan nutrien silaj rumput Napier, berbanding dengan penggunaan kaedah konvensional. Rumput Napier (*Pennisetum purpureum*) telah dicincang secara mekanikal dan satu bahagian darinya diperam di dalam tong plastik biru konvensional untuk tujuan fermentasi secara anaerobik; manakala baki satu bahagian lagi seberat setengah tan disiram dengan bancuhan tiga jenis probiotik dinamakan BIOBAC 1, 2 dan 3, yang mengandungi bakteria, fungus dan enzim. Probiotik telah dibancuh dengan air biasa, pada dos 100 mL per tan bahan bagi setiap jenis, dan dimasukkan ke dalam tong penyembur *haversack* dan disembur serta digaul rata ke dalam bahan. Bahan kemudiannya dikumpul setempat dalam bentuk busut di dalam bangsal berlantai konkrit dan dibiarkan selama 21 hari sehingga menapai dan matang. Cuma dalam masa lima hari pertama sahaja timbunan perlu sentiasa dilembabkan, untuk membolehkan percambahan fungus, dengan cara menyiram dengan air biasa keatasnya bila perlu. Seterusnya timbunan dibiarkan untuk matang dan kering. Silaj konvensional adalah disediakan dengan menggunakan kaedah fermentasi anaerobik di dalam tong plastik biru konvensional dan kemudian juga dibiarkan matang dalam masa 21 hari. Selepas 21 hari, sampel jerukan daripada kedua-dua jenis silaj diambil, dikering dan dianalisis bahan kimia di makmal mengikut kaedah *Association of Official Analytical Chemist* (AOAC) untuk perbandingan. Analisis proksimat kedua-dua jenis silaj telah dijalankan di Institut Veterinar Malaysia, Kluang, Johor. Pada silaj probiotik; peratusan kandungan bahan kering, protein kasar, lemak kasar, serabut kasar, abu, kalsium, forforus, ekstrak bebas nitrogen, jumlah nutrien tercerna, tenaga termetabolisme dan pH ialah masing-masing 65.06%, 9.50%, 0.84%, 42.72%, 6.92%, 0.18%, 0.16%, 40.06%, 49.48%, 7.25 MJ/Kg dan 6.82%, secara berurutan. Manakala untuk silaj konvensional; kandungan nutrien ialah 23.40%, 6.26%, 1.92%, 49.56%, 3.96%, 0.08%, 0.09%, 38.26%, 42.22%, 6.06 MJ/Kg dan 5.51%, secara berurutan. Kandungan nutrien silaj probiotik didapati lebih tinggi secara bererti berbanding kandungan nutrien silaj konvensional. kajian ini menunjukkan, kaedah probiotik telah memperbaiki sebahagian sahaja kandungan nutrien silaj Napier terutamanya protein dan tenaga. Pembaikan kandungan EE, CF, abu dan pH adalah tidak bererti, yang menunjukkan bahawa kaedah probiotik tidak dapat memperbaiki kandungan semua nutrien silaj. Oleh itu, probiotik adalah alat alternatif untuk penghasilan silaj yang berpotensi di Malaysia, yang menghasilkan sifat tambah-nilai dalam silaj yang dihasilkan, terutama protein dan tenaga, iaitu nutrien yang terpenting yang diperlukan dalam makanan haiwan.

Kata kunci: Silaj konvensional, probiotik, fermentasi, analisis proksimat

INTRODUCTION

In Malaysia, as in many humid tropical countries, green forages are plentiful for most parts of the year. The problem is farmers lack the time to cut the grass, especially during the main crop-planting period and harvesting season and also during major festive and religious events (Wong, 2000). However, at times, such as during a drought, livestock farmers will experience a shortage of forages and feeding of ruminant livestock will become a problem. Production of feed dry matter (DM) can be reduced tremendously during prolonged droughts, whilst during excessive rainfall, flooding can affect forage availability, harvesting and transportation. Fodder conservation seems to be the option to ensure feed availability during periods of feed limitation (Mohd Najib *et al.*, 1993).

Forage, crop residues and by-products are usually consumed fresh by domestic animals. However, it is possible to conserve them for use during those periods of feed shortages. Conservation can be achieved by sun drying (hay), artificial drying and addition of acids or fermentation (silage) (`tMannetje, 1999). Silage making is less dependent on weather conditions than hay making. Besides that, silage conservation no longer needs to be labour-intensive like cutting grass every day for ruminants (Wong, 2000).

Very recently there are new methods of producing silage, for example by using probiotics. Probiotics are live microbes which beneficially affect the host animal by improving its intestinal microbial balance (Parker, 1974). The most commonly used organisms in probiotic preparations are the lactic acid bacteria (lactobacilli, streptococci and bifido bacteria). These are found in large amounts in the gut of healthy animals and do not appear to affect them adversely. Organisms other than lactic acid bacteria, which are currently being used in probiotic preparations, include *Bacillus* sp., yeasts (*Saccharomyces cerevisiae* and *S. boulardii*) and filamentous fungi (*Aspergillus oryzae*). These microbial species are nowadays being prepared and used for the fermentation of fibrous feed materials to produce silage, instead of them digesting the fibre in the rumen. By using these microbes, silages can be prepared by different techniques, including the most recent method mentioned. In this paper, our current knowledge on general silage microbiology is reviewed with the aim of assisting in the choice of the best ensiling strategy to produce high quality silage to assist animals during adverse periods, to replace fresh forages.

Aminah *et al.* (2000) had studied the composition and ensiling characteristics of several tropical grasses and forage crops in Malaysia. The *Setaria* and Napier grasses have produced acceptable silages. That is why in this study, Napier grass (*Pennisetum purpureum*) was used to prepare silage which has become by far the most important grass due to its wide ecological range, high yield and ease of propagation and management. Moreover, Napier grass is best suited to high rainfall areas, and it is drought-tolerant too and can grow well in drier areas, which are suitable to our climate. It does not grow well in waterlogged areas. It can be grown along with other crops along field boundaries or along contour lines or terrace risers to help control erosion. It can be intercropped with crops such as legumes and fodder trees, or as a pure stand. It has a soft stem that is easy to cut. It has deep roots, so is fairly drought-resistant. The tender, young leaves and stems are very palatable for livestock and it grows very fast.

Despite the two extreme weather conditions, *i.e.* drought and flood, there is still a need for silage making under local conditions, especially in those areas experiencing drier months or where monsoonal conditions persist, which restrict the routine cutting of forages (Aminah *et al.*, 2000). There are many benefits that can be obtained from this study especially to find the best and the most cost effective method of preparation of silage in order to increase animal production and to improve our livestock industry. This study is intended to compare the different methods of silage production from Napier grass. It is to investigate whether the probiotics method, which uses aerobic conditions, can increase the nutrient values of Napier silage. Besides that, this new technology would be able to help farmers cut the cost of silage preparation in managing their pasture more effectively and efficiently so that they would be able to gain more profit.

In Malaysia, farmers face a lot of problems regarding forage for their livestock feeding. Some farmers do not have land to plant grass but they have a lot of livestock to be fed. One of the major constraints to ruminant production is limited availability of land for grazing or planting grass (Halim, 1996; Kayouli and Lee, 1999). With limited land resources for forage production, it is especially important to produce higher yielding and high quality forage. In addition, many species of tropical forages are low in nutritive values, which thus need to be made into silage, as it cannot sustain high animal productivity because of low metabolizable energy content. A new method of silage making is needed to overcome this problem (Wong, 2000).

The preparation of silage by the probiotic method is not yet well publicized compared to the conventional plastic drum method as it is a new technology and lacks dissemination of information. The preparation of silage by the probiotic method does not need anaerobic conditions. The farmer only needs space with a shed and to maintain the humidity of the silage in order for the microbes to work efficiently. These methods also result in farmers no longer having to use plastic drums like the traditional method. Problems with the drum method arise when the drum is opened for the silage to be fed to the animals, as it needs to be finished on the same day, due to spoilage caused by molds that grow particularly fast at the high temperatures once the drum is opened. This is a common problem in the tropics. Therefore, special ensiling technology should be developed for such farms to meet their needs and to be economically feasible. By using the probiotic method, the silage would still be in good condition when parts of it are taken out for use, as it is dry when it matures. It eases storage and transportation, and can be mixed with feed concentrates to form total mixed feed (TMF).

The objectives of the study were: (1) to compare the best method of silage production between conventional and probiotic method in terms of the preparation procedures; (2) to differentiate the nutritive values of silages from the two different methods which are the conventional or drum packaging and the probiotic method; and (3) to identify other advantages and disadvantages of both methods.

MATERIALS AND METHODS

Half a tonne of chopped Napier grass was made ready for the two types of ensiling. Other materials and equipment needed were: plastic drums of 60 kg capacity, a shed with concrete flooring, haversack sprayer, shovels, enough water, probiotic sets of three bottles, made up of BIOBAC 1, 2 and 3, containing bacteria, fungus and an enzyme.

The Napier was harvested at six weeks of age and let to wilt for several hours because too much water in the forage can spoil the silage. After that, the Napier was chopped into small pieces in order to be able to be compacted well into the plastic drum, to ensure a well prepared silage was produced.

Conventional Silage

In the conventional method, Napier was placed into the blue plastic drum and compacted manually. Conventional silage was formed through anaerobic fermentation by microorganisms. It needs to be ensured that all air is excluded to prevent entry of oxygen that could kill the anaerobic bacteria that was supposed to help in the fermentation process before the container lid was closed air-tight. It was kept as such for 21 days to ensure complete fermentation.

Probiotics Silage

For the other ensiling process, the probiotic method, the chopped Napier grass was spread thinly on the concrete floor housing without walls. Approximately 40 mL each of the probiotics, BIOBAC 1, 2 and 3, consisting of bacteria, fungus and an enzyme solution was mixed thoroughly into a large pail of tap water, enough to be sprayed over the 400 kg of Napier. The mixture of the water and probiotics was filled into a haversack sprayer and sprayed onto the Napier grass. The grass was then mixed several times using a shovel so that the solution covered all the ensiling material. They were then shaped like a mount on the concrete floor and were left uncovered. This was to allow for air and moisture to seep in, to make sure the bacteria and fungus were kept alive. This was also kept for 21 days to mature. On the first five days it had to be ensured that the silage was not too dry, and if it became too dry, plain water had to be sprayed once over the top.

Both types of silages were sampled after 21 days. Five spots were selected at random for each type, samples were taken and they were sent to the laboratory. Each spot taken was considered a sample and the number of samples for each type was thus five. Complete proximate analysis was done on both silage types.

Proximate analysis was done to determine contents of dry matter (DM), crude protein (CP), ether extract (EE), crude fiber (CF), ash, Calcium, Phosphorus, Nitrogen free extract (NFE), total digestible nutrients (TDN), metabolizable energy (ME) and pH according to the Association of Official Analytical Chemists (AOAC, 1975) method, and conducted at the Malaysian Veterinary Institute (IVM), Kluang, Johor, Malaysia.

Paired *t*-test analysis was used to determine the significant difference of means of the composition of chemical nutrients in both the silage types.

RESULTS AND DISCUSSION

Comparison of nutrient content of both methods

The data obtained from proximate analysis were analyzed statistically using the independent *t*-test. The results were presented in Table 1.

The results showed that the mean value of dry matter (DM) of the conventional method was significantly different from the probiotic method (Table 1), while the mean of crude protein (CP) of the conventional method was significantly lower ($p < 0.05$) than the probiotic method. On the other hand, the mean of EE, CF, ash and phosphorus (P) of the conventional method were not significantly different from the probiotic method ($p > 0.05$). The mean of calcium (Ca), NFE, TDN and ME for the conventional method were conversely significantly different from the probiotic method with means for the probiotic method being higher than the conventional method for all the parameters ($p < 0.05$).

Table 1. A comparison of nutrient content between conventional silage and probiotic silage.

Treatments / Parameters	Conventional	Probiotic	*t*-value
DM (%)	23.40(± 0.59)^{a}	65.05(± 7.29)^{b}	5.70
CP (%)	6.26(± 0.10)^{a}	9.50(± 0.60)^{b}	4.10
EE (%)	1.92(± 0.20)^{a}	0.84(± 0.10)^{a}	4.83
CF (%)	49.56(± 2.87)^{a}	42.72(± 1.39)^{a}	2.14
Ash (%)	3.96(± 0.07)^{a}	6.92(± 0.30)^{a}	9.76
Ca (%)	0.08(± 0.04)^{a}	0.18(± 0.02)^{b}	4.95
P (%)	0.09(± 0.01)^{a}	0.16(± 0.03)^{a}	2.37
NFE (%)	38.26(± 3.00)^{a}	40.06(± 1.37)^{b}	3.80
TDN (%)	42.22(± 2.87)^{a}	49.48(± 1.12)^{b}	2.36
ME (MJ/kg)	6.06(± 0.47)^{a}	7.26(± 0.18)^{b}	2.37

Note: *a, b means in the same row having different superscript was significant ($p < 0.05$). Number of samples analysed from conventional method was 5 and from probiotic method was 5.

The pH value was measured separately during the lab analysis. The mean of pH values of conventional silage and probiotic silage was presented in Table 2.

Table 2. A comparative mean of pH value between conventional silage and probiotic silage.

Treatment	Mean of pH value
Conventional	5.51
Probiotic	6.82
t-value	4.79

Fodder conservation has the main objective of ensuring feed availability during periods of scarcity of feed supply (Mohd Najib *et al.*, 1993). Silage production is one of the conservation methods in order to maintain the supply of feedstuff for livestock. Besides, it can be used to maintain, and in some cases even can increase the nutritive value. It is practiced widely among Malaysian farmers in order to increase feed quality at any time of the year to complement grass for the nitrogen utilization and help to improve animal production. Preservation of forage crops by conventional silage production in the tropics might be problematic as it cannot be done in the rainy season. If the harvest is postponed to the beginning of the dry season, the nutritive value of the forage can decrease considerably, and become much less digestible (MacLaurin and Wood, 1987). In this study, Napier grass (*Pennisetum purpureum*) was used due to its high yielding fodder with one of the most promising fodder species available (Anindo and Potter, 1994) and with dry matter yields that surpass most tropical grasses (Skerman and Riveros, 1990; Humphreys, 1994).

This research program undertaken was to investigate a new method of silage production, which is the probiotic method. Parker (1974) defined probiotics as organisms and substances which contribute to intestinal microbial balance. Subsequently, Fuller (1989) modified it into live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance. Probiotics are generally known as the good bacteria that can help to stimulate the nutritive value and help livestock to fight illness and disease (Fuller, 1989). He further iterated that they were originally incorporated into feed to increase the animal's growth and to improve its health by increasing its resistance to disease.

The probiotics silages were prepared by spreading chopped Napier grass on a cemented floor, under the shed without walls as practiced by Adnan Atob (2010). The probiotics used were namely BIO-BAC 1, BIO-BAC 2 and BIO-BAC 3, consisting of bacteria and fungi. A previous study by Shinoda (1999) on conventional silage showed that the reported nutritive values, especially of DM and CF, are lower when compared to our present study, while the CP, EE, and NFE of his study are higher. This may have been due to several factors such as weather, harvesting age and ensiling technique.

As defined by Van Saun (2006), dry matter is the non-moisture portion of a feed ingredient. Dry matter contains the essential nutrients and varies widely within a given feed ingredient or forage. The lower the DM, the more moisture present, and the lower the nutrient density in the fresh feed. Also, high moisture may decrease the keeping quality of a feed unless it is made into silage. From our result, the mean of DM of conventional silage is 23.40% while probiotic silage is 65.06%, respectively. This shows that the probiotic silage was drier when matured compared to conventional silage. It also can be kept longer and does not get damaged even though some of it is taken for use at any one time.

For crude protein (CP), the mean obtained for the probiotic silage was 9.50%, which was higher than the conventional silage (6.26%). Crude protein content is often considered a good determinant of silage quality. CP is an estimate of the level of protein in the feed based on the amount of nitrogen present. Since only some of the nitrogen is in the form of true protein, it is termed "crude" protein. As stated by Van Saun (2006), protein cannot be directly measured and it is estimated from feed sample nitrogen (N) content. On average all biological proteins contain 16% N, therefore protein content was estimated by multiplying N% by 6.25 resulting in 0.16% of protein content.

Ether extract, also termed as crude fat, is a chemical compound in which all lipid (fat) soluble compounds are contained. Crude fat (CF) is a heterogeneous material, consisting of a mixture of triacylglycerol, phospholipids, fatty acids, sterols, waxes and pigments (Xiao, 2010). Based on the results, the ether extract of conventional silage was higher compared to probiotic silage, which was 1.92% and 0.84%, respectively.

Crude fat is the insoluble carbohydrate remaining in the feed analysis process after the sample is boiled in weak acid and alkali. The CF value of probiotic silage was 42.72% and conventional silage was 49.56%, which is higher than the probiotic silage. The probiotic silage is thus better since it has a low CF value, because forage feeding values are negatively associated with fiber, since the less digestible portions of plants are contained in the fiber fraction (Linn and Martin, 1999).

The ash content for probiotic silage was 6.92% whereas for conventional silage it was 3.96%. The ash content is a measure of the total amount of minerals present within a food, whereas the mineral content is a measure of the amount of specific inorganic components present within a food (McClements, 2007). In this study, we went further to measure the calcium and phosphorus content, which were 0.08% and 0.09% for conventional silage and 0.18% and 0.16% for probiotic silage, respectively. Ash is not digestible by animals. High ash content of feeds may dilute the amount of nutrients available to the animal (DuPonte, 2007).

According to the results, the NFE of the two methods were almost similar with 38.26% (conventional silage) and 40.06% (probiotic silage). NFE consists of carbohydrates, sugars, starches, and a major portion of the hemicellulose in feeds. It is calculated when crude protein, fat, water, ash and the fiber are added, and the sum of it is subtracted from 100. For TDN, the value was 42.22% for conventional silage and 49.48% for probiotic silage. Proper preparation of silage minimizes the loss of nutrients during the fermentation process and increases voluntary intake, which results in higher TDN intake (Shinoda, 1999).

Van Saun (2006) wrote that energy content is often used to compare and evaluate quality. Feed energy content is not directly measured like other nutrients but derived through regression equations, while metabolizable energy (ME) means what is left after accounting for energy in faeces, urine and gasses. It is still not all available for the animal to use. When comparing both methods, the ME for the probiotic method (7.25%) was significantly different from the conventional method (6.06%).

Finally, pH is a useful method for the evaluation of silage quality especially in developing countries where the analysis of lactic acid is not practical in terms of cost and facilities (Shinoda, 2000). The value of pH itself may also be a useful and very simple indicator for the evaluation of silage quality. The pH for probiotic silage is not significantly different from conventional silage since both are nearer to neutral. Low pH in silages is often associated with poor intakes because low pH in the rumen will reduce cellulolytic activity and depresses intake. However, there is no relationship between silage pH and rumen pH (Rooke, 1995), because silage is neutralized by saliva upon consumption. However, Rooke (1995) also suggested that lactic acid may have a direct effect on palatability, since sour taste is associated with reduced palatability.

CONCLUSION

This study was to describe the effects of a new technology in silage making which used probiotics to increase the nutrient content of the silage. Proximate analysis was carried out to analyze the nutrient content of conventional silage as well as those of probiotic silage. In conclusion, there is an enormous potential for the use of probiotics in farm animal feed and with probiotic, we need to know more about the fundamental mechanism of probiotic activity. The probiotic effect of silage inoculation on rumen fermentation has been proposed to explain improved animal performance from inoculated silages, in the absence of changes in silage fermentation. Modern ensiling technology has proven that it can increase the feeding value of silages close to that of the original unensiled forage. The study arrived at the expected results as it was proven that probiotic silage has high potential to be used to increase forage nutritive value.

The effectiveness of silage production will undoubtedly contribute to improvements in animal production due to quality feed availability. The possibility that in the future, silages will add superior feeding value to the original crop is realistic. The present study has proven that probiotic silage was able to increase nutrient content.

ACKNOWLEDGEMENTS

The authors wished to thank the Head of School of Animal Science, Faculty of Bioresources and Food Industry for his kind encouragement and support to carry out the trial project. Thanks were also due to the officers and staff of the Veterinary Institute of Malaysia (IVM), Kluang, Johor; for helping to carry out the chemical analysis of all the feed samples, and all those who had helped in one way or another to make this trial project a success.

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