Silage without Supplementation: Problems, Advantages and Lactic Acid Bacteria Green Approach

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***Abstract*— This article review started with some techniques which can indicate the quality of silage such as sensory evaluations (contour of bunker face, color and odor), chemical compositions (moisture, crude protein, soluble protein, ammonia nitrogen, acid detergent insoluble nitrogen, neutral detergent fiber, acid detergent fiber, fermentation profiles and mould counts and mycotoxin concentrations) and physical characteristics (pH, temperature and particle size). But, most effective indicator is the nutritions quality. It was a key factor to reduce the production cost of feeding of ruminants. There are a lots of nutrients losses especially protein content of forage during fermentations. Lots of losses have been occured during aerobic phase and feedout. Most of the value of protein degradation rate is around 0.5-1.0% per day which gives bad performance especially at the end of fermentation result. There are two chemical process occurs which are Maillard reaction and acid hydrolysis of hemicellulose which both can effect silage quality especially the nutrient loss through degradation and utilization. Lactic acid is preferred to be dominant over other silage fermentation acids because it has a lower dissociation constant (Ka=3.86) which can be major organic acid responsible for decreasing silage pH. Lactic acid bacteria application so-called as green application will give 20 gram of lactic acid production per kilogram compare to control that resulted almost neal lactic acid. This acidic condition will help to stabilize the silage through green low pH condition instead of apply chemical like strong acid which give negative impact and in the same time can reduce the nutrient loss along fermentation and storage.**

***Index Terms*— Silage, Animal feeds, Nutrient loss, Lactic acid bacteria, Advantages**

I. Introduction

In some countries, silage produced from agricultural wastes has gradually replaced expensive source of animal feeds Besides, silage can be preserved forages and it is convenient for countries that have four seasons where the farmers will get ready the livestock feeding during winter season [1]. Therefore, silage can be classified as a sustainable product because of its ability to reproduce from waste of plant and animal. Some factors that must be considered are the selection of alternative feeds for cows or other animals, such as supplementation description, availability and storage, feeding and limitation, nutrient density and mineral supplementation [2]. Ensiling process can be defined as a preservation of moist forage crops under anaerobic condition to enhance the nutrient content. As a result, the pH value will decrease and the moist forage is preserved from spoilage

(pH, temperature and particle size) [4]. But, most effective indicator is the nutritions quality. It was a key factor to reduce the production cost of feeding of ruminants. Various studies have explored in dry matter losses in silage which occurs hen aerobic microorganisms

This work was supported in part by the Universiti Teknologi Malaysia under Grant Q.JI3000.7125.00H02.

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ISBN: 978-988-19252-8-2
ISSN: 2078-0958 (Print); ISSN: 2078-0966 (Online)

decompose the readily available carbohydrate and this will reduce the carbohydrate content for utilisation [5].Besides, high nitrates content of silage also indicate the nutrient loss during fermentation. Nitrates will harm to human and livestocks. It will produce from the slow fermentation rate and if the level exceeded, it will be toxicity and poisoning to ruminants.

For instance, 25 acres of corn silage harvested and it will yield about 20 tons per acres. 500 tons available to be feed over the next 12 months. But, will there enough amount of silage production available for the next year feeding? It was impossible to get the same amount based on dry matter losses, wastage and spoilage. Therefore, the loss must be reduced to fulfil the nutrient requirements of ruminants. In facts, to achieve the good manufacturing practise (GMP) in silage production, the silage loss must be low and it will improve the silage quality simultaneously. The current average of dry matter losses is about 22% [6].The percentage of dry matter loss during harvesting is (2-12%), respiration and fermentation (5-18%), silage effluent (0-8%) and during aerobic deterioration (1-10%).

Table I shows the nutrient loss of various types of forages before and after ensiling without any supplementation or additives. There are a lots of nutrients losses especially protein content of forage during fermentations. A lots of losses has been occurs during aerobic phase and during feedout. Proteolysis is the process of protein degradation into amino acids, ammonia and peptides by enzymes named protease and peptidase. During feed out, largest losses dry matter and nutrient occurs caused by an aerobic microbe consuming sugar and fermentation product like protein. Most of the value of protein degradation rate is around 0.5-1.0% per day. It shows that the type of forages does not affect the rate of protein utilisation by microbes.

Besides, the value of pH level is closely related to the lactic acid produce in silage but it also depends on the buffering capacity of type of forage use. For instance, forage with high buffer capacity will give low amount of lactic acids to achieve some level of pH compare to the low buffering capacity forages. In fermentation phase, all microbiology strain like Lactic acid bacteria, clostridia, yeasts and molds compete each other to utilize carbohydrate and other nutrients. Low pH level will inhibit some strain to be survived and in the same time it can reduce the nutrients loss in silage.

III. Advantages of Ensiling Process Instead of Anaerobic Solid State Fermentation on Maintaining The Nutrients in Silage

Solid-state fermentation so called as absence or near absence of any fluid in the space between particles of forage during fermentation. The liquid phase is represented by the aqueous film that surrounds the cell while the space between particles is occupied by the gas phase. In solid phase, the proportion of each phase depends on the type of substrate that is being used. Solid medium can be considered more heterogeneous in term of the microbial population and solute concentration. Higher heterogeneity will give less accurate result [13]. The solid material will act as physical support and source of carbon and nutrient to sustain microbial growth.

Ensiling is a combination of anaerobic condition and forage's acidity protection from proliferation of deleterious bacteria and fungi. Instead of using solid-state fermentation, ensiling can give high palatability due to lactic acid production, which suitable to produce high quality of ruminant feed [14]. It also apply on producing clean energy as an alternative for energy source where the methane produce in silage is around 0.253 m3/kg total solid [15].Ensiling process are suitable in producing juicy livestock with water content more than 50% compared to solid state fermentation and in the same time can improve the heterogeneity.

During ensiling process, there are two chemical processes occur which are Maillard reaction and acid hydrolysis of hemicellulose that both can effect silage quality without good controlling [16]. Maillard reaction is the browning reaction where sugars react with amino acids and releasing heat. In making silage, excess heat causes the Maillard reaction to occur, which reduces the amount of energy and protein available to the animals who feed on it. It will form large molecule that are slowly digestible. The rate of reaction is slow and does not substantially affect silage quality when temperature is below 100°C. Under extreme condition where the moisture content is below 40%, heat given off by the process that can raise silage temperature and starting silo fires in dry silage.

TABLE I
Nutrient Losses In Various Types of Silage and Its pH Values

	% of losses / days	pH	Lactic (g/kgDM)	Butyric (g/kgDM)	Acetic (g/kgDM)
Crude protein in bamboo shell [7].	43/90	5	4	22	32
Crude protein in wheat [8].	26/30	5	7	5	8.0
Organic matter in Mucuna [9]	18.9/7	-	-	-	-
Crude protein in bamboo shot shell [7].	32/40	5	38	-	13
Crude protein in wormwood [10].	51/90	4	4.81	0.07	1.02
True protein in regular millet [11]	29/45	7	2.49	-	-
Crude protein in Soyabean [12]	25/30	5	1.98	16.5	3.82

ISBN: 978-988-19252-8-2
ISSN: 2078-0958 (Print); ISSN: 2078-0966 (Online)

For second chemical process, it is named acid hydrolysis of hemicelluloses, which involves the slow process of chemical breakdown of hemicelluloses in the plant cell wall. It is caused by interaction with hydrogen ions in the silage. If the pH declined, the hydrogen ion concentration will increase faster (faster the rate of hydrolysis). It will reduce Natural Detergent Fiber (NDF) content but in slow reduction. Acid-catalyzed hydrolysis is a complex heterogeneous reaction, involving physical factors, as well as the hydrolytic chemical reaction.

In general, the crop preserved in the silo by two factors, an anaerobic (oxygen free) environment and low pH condition. An anaerobic condition is important to prevent the growth of spoilage microorganisms, which need oxygen. Minimum pH condition is required to inhibit detrimental anaerobic microorganisms and plant enzyme activity. Lactic acid bacteria on the crop will ferment sugars to lactic acid. Once anaerobic condition occurs, many plant cell will rupture within hours and release many enzyme including proteases process where protein will breakdown to soluble non-protein fraction that must be avoided and hemicelluloses breakdown into sugar using acid produce during fermentation or as an addition.

Besides, other ensiling condition like selection of suitable silo and exclusion of oxygen in bunker silo are also very important to produce good silage. To select the best silo, some factors such as oxygen exclusion, packing density and less affected by weather must be considered. Bunker silos, tower silos, plastic tube and plastic bag are the common silo used in ensiling process. Bunker silo is cheaper to construct rather than tower but it suffers from higher dry matter losses and least forgiving of errors in silage management. Tower silo is expensive but high quality of silage can be produced easily same like plastic tube. Plastic bag will give low dry matter losses but it is economical because for 1 ton, 9 feet diameter of plastic bag is used. However, it must be inspected from hole.

After selecting the best silo, the exclusion of oxygen in bunker silo must be considered. To ensure that, the packing of the silage to an optimal density and ensure the sealing of the packed with atmospheric barrier like polyethylene sheets by increasing the density, reduce the porosity and reduce the penetration of air into silage mass. The density depends on the type of implement during packing and the total time spent back per ton. To get the maximum impact of packing method, the tires must apply the greatest weight per unit of surface area. Several factors influence the total time spent to pack such as forage delivery rate, number or weight of packing tractors and total time spent during packing. Based on normal application by industry, the optimum packed rate is 1 to 4 minutes per ton of forage and the optimum packing layer is 15 centimetres or less. The chopping length of forage is not related to the packing density and the minimal density of packing is 225 kg DM/m3. Besides, it was proven that temperature and chopped length do not significantly affect the quality of silage produced but the fermentation period must be considered as a crucial parameter to produce high quality of silage [17].

IV. Lactic Acid Bacteria Application in Silage Without Any Supplementation

Lactic acid bacteria are the group of bacteria that produce lactic acid from fermentation of carbohydrate. Function of lactic acid bacteria in plant is to protect from pathogenic microorganisms by producing some antagonistic compound like organic acid and antifungal agents [18] Lactic acid is preferred over other silage fermentation acids because it has a lower dissociation constant (Ka=3.86) where can be the major organic acid responsible for decreasing silage pH [12].More than 9 mg/g of fresh material is a good lactic acid indicator for good silage production and it shows the absence of butyric acid. The success of inoculant in silage depend on many factors such as type and properties of the plant to be ensiled, climate condition, epiphytic microflora, ensiling technique and properties of inoculant [3].

Other than that, the criteria to choose the suitable lactic acid bacteria strain is based on the ability to dominate the fermentation, potential synergetis activity, ability to enhance animal performance by altering ruminal fermentation. The concept of adding lactic acid bacteria is fast growing as it dominates the fermentation due to many detrimental types of bacteria presented in it such as clostridia as well as yeast and mold present. It was because of the viable lactic acid baceria population attached to the plant is not enough to enhance lactic acid bacteria dominant fermentation (less than 102 cfu/g) [19]

Microbiology could be applied in many forms like powder, granular and fresh culture but fresh culture was the best application because bacteria would have shorter lag time for being revived from freeze dried state [20].For applying the fresh culture, the moisture content can be the best factor on their performance. Bacteria can have different tolerance against low water activity. According to a study conducted by Pieper [21], inoculant apply in low water content of forage will give 20 gram of lactic acid per kilogram product compare to control that resulted almost no lactic acid. For high moisture content forage, the osmotolerant bacteria must be choosen as an inoculant that can reduce yeast activity.

Undesirable characteristic of each type of microbial additive may be overcomed by combining them in a silage inoculant. Some studies have combined two or more strains of microbiologies for some function and reduce nutrient content as in the Table II. It is shown that homofermentative bacteria is dominating the combination with heterofermentative bacteria in silage.

ISBN: 978-988-19252-8-2
ISSN: 2078-0958 (Print); ISSN: 2078-0966 (Online)

TABLE II
EFFECTS OF COMBINATION OF TWO AND MORE STRAIN OF MICROBIOLOGIES IN SILAGE

Combination	Effect	Reference
Lactobacillus bucheneri with *Lactobacillus plantarum*	Dry matter loss were reduced	[22]
Lactobacillus bucheneri with *Pediococus pentoseeus*	Not consistent effects aerobic stality	
Lactobacillus bucheneri and *Lactobacillus plantarum*	Higher concentration of lactic acid compare to control	[23]
Lactobacillus plantarum and *Lactobacillus acidipropionic*		

V. POTENTIAL OF HOMOFERMENTATIVE AND HETEROFERMENTATIVE LACTIC ACID BACTERIA IN SILAGE

Lactic acid bacteria can be divided into two groups, namely homofermentative and heterofermentative based on the function and characteristic in fermentation process. Almost all bacterial species belong to heterolactic bacteria, which resulted acetic acid and carbon dioxide after fermentation process [21]. Heterolactic bacteria can increase stability of silage against deterioration by yeast and mold when exposed to air [18] by accumulation of acetic acid [11, 20]. Heterolactic can be divided into two sub-groups which are facultative and obligate. Facultative will ferment hexoses into lactic acid under special condition. It will produce lactic acid, carbon dioxide, ethanol or acetic acid. For instance, *Lactobacillus alimentarius, Lactobacillus casei, Lactobacillus curratus, Lactobacillus sakei, Lactobacillus paramenterius, Lactobacillus plantarum and Lactobcillus pentoses*. Heterolactic obligate will ferment hexose to lactic acid, carbon dioxide and ethanol or acetic acid in the presence of alternative electron acceptor. The members of this group are *Lactobacillus Brevis, Lactobacillus bucheneri, Lactobacillus fermentum, Lactobacillus reuti, Lactobacillus fuctinocus, Lactobacillus sanfraciscensis and Leuconostoc mesenteroides* (Holzer *et al.*, 2003). Lactobacillus bucheneri is the best choice for silage fermentation process due to the ability to form acetic acid from lactic acid that is responsible to increase silage stability. Combining Lactobacillus bucheneri and a homolitic acid bacterium will reduced fermentation losses compared to buchneri alone [22].

Homolactic bateria produce more than 850gram per kilogram lactic acid from fermentable sugar. It will produce two molecules of lactic acid and reduce acetic acid, butyric acid, ammonia nitrogen and pH [11, 20, 23] It will also enhance aerobic deterioration by act as energy sources of yeast and molds and probably because of inadequate volatile fatty acid to inhibit fungus [3, 23]. Homofermentative obligate will convert hexose into lactic acid via Embden Meyerhof Pathway such *as Lactobacillus acidophilus, Lactobacillus delbrueckii, Lactobacillus facimnis, Lactobacillus lactic* and *Lactobacillus bovis* [18]. The use of homofermentative bacteria inoculant might lead to such condition in some sugar rich silage, since the homolytic fermentation is more efficient and utilities less water soluble carbohydrate than heterolactic fermentation, which can result in a higher content of residual sugar and lactic acid in silage. The presence of low concentration of oxygen in silage result in a shift of homolactic fermentation to heterolactic metabolisms. The simultaneous production of acetic acid and lactic acid together with low water soluble carbohydrate content constitute the most important factors to reach stability in silage. Acetic acid is stronger antimytotic than lactic acid that acts as inhibitor on spoilage microorganisms by decrease of the maximum growth rate, acetic acid increases the aerobic stability exponentaly. Until recently, acetic acid was primarily associated with the activity of clostridia (not desirable in silage).

VI. EFFECTS AND DISTRACTION OF INOCULATION LACTIC ACID BACTERIA IN SILAGE

The application of inoculants lactic acid bacteria in silage was the new finding in biotechnology industries. Due to the aim of producing a good quality of silage that has lowest pH created from high amount of lactic acid, homofermentative lactic acid is more suitable but silage must also have aerobic stability characteristic that can be produced by heterofermentative lactic acid. Therefore, the positive and negative effects and the distractions must be clearly defined to avoid the mistake during making choices of the best bacteria for silage. Positive effects of inoculants lactic acid bacteria as studied by [22] resulted similar aerobic stability among inoculated silage (more than 500 hours) which is more stable than control (180h).

Originally, forage containing epiphytic lactic acid bacteria that will convert free sugar into lactic acid and the use of starter cultures of lactic acid bacteria to produce high quality of feed. Besides, inoculated corn silage produce 0.7 kg per day more feed consumption rate than did cows fed control silage [20]. The other finding is the increasing rate of pH decline in the silo thereby decreasing ammonia-nitrogen concentration and preserving forage protein [11, 24]. This enhances the will to reduce proteolysis resulting higher proportion of retained protein. Figure 1 shows the sharp decline on water-soluble content in inoculated silage that makes pH declined faster than control. Inoculated silage reduces the incidence of diarrhea and increased body weight gain by 7% in first trial and 17% in a second time [25].

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ISSN: 2078-0958 (Print); ISSN: 2078-0966 (Online)

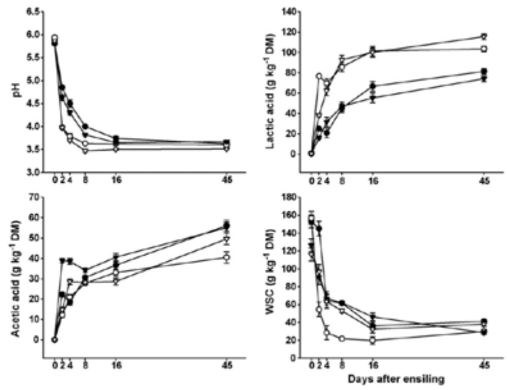

Fig.1: Kinetic study of pH, Lactic acid, Acetic acid and Water soluble carbohydrate, WSC of inoculated silage [11].

There are some distractions of using lactic acid bacteria in silage application especially when it is exposed to air. Inoculation makes silage less stable, produce mold and yeast as well as it leads to a small dry matter loss [11,22]. High possibility of proteolisis process become enhanced when high pH produce, no significant effect on degradability of corn silage and lower dry matter intake occurs due to acetic acid ester in *Lactobacillus bucheneri* contribute chemical flavor [23]. A study conducted by Bayatkouhsar *et al*, [20], it has been found that inoculation had no significant effect on dry matter intake and digestibility on body weight.

Acknowledgements

The Universiti Teknologi Malaysia is thankful for the grant given to Dr. S.H. Mohd-Setapar's project (Q.JI3000.7125.00H02) under the Research University Grant Scheme, part of which enabled this article review to be prepared. We are grateful for the contribution of all the people involved in this paper preparation.

REFERENCES

[1] Z.G. Weinberg,"The effect of sewage irrigation on safety and hygiene of forage crops and silage" *Animal Feed Science and Technology*, 2004. 116(3–4): p. 271-280.

[2] S. Gadberry, "Alternative Feeds for Beef Cattle "*Agriculture and Natural Resources*, 2007.

[3] I. Filya, "The effect of bacterial inoculants on the fermentation and aerobic stability of whole crop wheat silage". *Animal Feed Science and Technology*, 2000. 88(1–2): p. 39-46.

[4] P.J. Van Soest, "Rice straw, the role of silica and treatments to improve quality". *Animal Feed Science and Technology*, 2006. 130(3–4): p. 137-171.

[5] R.D. Watters, "Effects of Dry Period Length on Milk Production and Health of Dairy Cattle" *Journal of Dairy Science*, 2008. 91(7): p. 2595-2603.

[6] W.F. Raymond, "The Nutritive Value Of Forage Crops", in *Advances in Agronomy*, N.C. Brady, Editor 1969, Academic Press. p. 1-108.

[7] S.-Q. Liu,, R. Holland, and V.L. Crow, "Purification and properties of intracellular esterases from Streptococcus thermophilus" *International Dairy Journal*, 2001. 11(1–2): p. 27-35.

[8] N. Reddy, and Y. Yang, "Biofibers from agricultural byproducts for industrial applications". T*rends in Biotechnology*, 2005. 23(1): p. 22-27.

[9] L.K. Adjorlolo,, K. Amaning-Kwarteng, and F.K. Fianu, "In vivo digestibility and effect of supplemental mucuna forage on treated rice straw degradation". *Small Ruminant Research*, 2001. 41(3): p. 239-245.

[10] S.C. Kim, "Influence of replacing rice straw with wormwood (Artemisia montana) silage on feed intake, digestibility and ruminal fermentation characteristics of sheep". *Animal Feed Science and Technology*, 2006. 128(1–2): p. 1-13.

[11] F. Hassanat, A.F. Mustafa, and P. Seguin, "Effects of inoculation on ensiling characteristics, chemical composition and aerobic stability of regular and brown midrib millet silages". *Animal Feed Science and Technology*, 2007. 139(1–2): p. 125-140.

[12] R.. Lima, "Effect of combined ensiling of sorghum and soybean with or without molasses and lactobacilli on silage quality and in vitro rumen fermentation". *Animal Feed Science and Technology*, 2010. 155(2–4): p. 122-131.

[13] C.R. Soccol, and L.P.S. Vandenberghe, "Overview of applied solid-state fermentation in Brazil". *Biochemical Engineering Journal*, 2003. 13(2–3): p. 205-218.

[14] J.-X. Liu,, X.-Q. Wang, and Z.-Q. Shi, "Addition of rice straw or/and wheat bran on composition, ruminal degradability and voluntary intake of bamboo shoot shells silage fed to sheep". *Animal Feed Science and Technology*, 2001. 91(3–4): p. 129-138.

[15] V. Benetka, F. Vrátný, and I. Šálková, "Comparison of the productivity of Populus nigra L. with an interspecific hybrid in a short rotation coppice in marginal areas". *Biomass and Bioenergy*, 2007. 31(6): p. 367-374.

[16] C. Devendra, and C.C. Sevilla, "Availability and use of feed resources in crop–animal systems in Asia" *Agricultural Systems*, 2002. 71(1–2): p. 59-73.

[17] S.H. Mohd-Setapar, N. Abd-Talib, and R. Aziz, "Review on Crucial Parameters of Silage Quality". *APCBEE Procedia*, 2012. 3(0): p. 99-103.

[18] M. Holzer, "The role of Lactobacillus buchneri in forage preservation" *Trends in Biotechnology*, 2003. 21(6): p. 282-287.

[19] E.K. Elmenofy, "Improving the nutritive value of ensiled green rice straw 1- Fermentation characteristics and chemical composition". J. *Agric. Res. Kafer El-Sheikh Univ*, 2011: p. 571-582.

[20] J. Bayatkouhsar, A.M. Tahmasebi, and A.A. Naserian, "The effects of microbial inoculation of corn silage on performance of lactating dairy cows". *Livestock Science*, 2011. 142(1–3): p. 170-174.

[21] R. Pieper, "Effect of ensiling triticale, barley and wheat grains at different moisture content and addition of Lactobacillus plantarum (DSMZ 8866 and 8862) on fermentation characteristics and nutrient digestibility in pigs." *Animal Feed Science and Technology*, 2011. 164(1–2): p. 96-105.

[22] L.J. Reich, and L. Kung Jr, "Effects of combining Lactobacillus buchneri 40788 with various lactic acid bacteria on the fermentation and aerobic stability of corn silage" *Animal Feed Science and Technology*. 2010. 159(3–4): p. 105-109.

[23] H. Mohammadzadeh, "Frosted corn silage with or without bacterial inoculants in dairy cattle ration". *Livestock Science*, 2012. 145(1–3): p. 153-159.

[24] Z.-K. Lee, "Effect of pH in fermentation of vegetable kitchen wastes on hydrogen production under a thermophilic condition". *International Journal of Hydrogen Energy*, 2008. 33(19): p. 5234-5241.

[25] H.M. Abu-Tarboush,, M.Y. Al-Saiady, and A.H. Keir El-Din, "Evaluation of diet containing Lactobacilli on performance, Fecal Coliform, and Lactobacilli of young dairy calves" *Animal Feed Science and Technology*, 1996. 57(1–2): p. 39-49.

ISBN: 978-988-19252-8-2
ISSN: 2078-0958 (Print); ISSN: 2078-0966 (Online)