Effect of lactic acid bacteria inoculants applications to the quality and chemical composition silage waste of carrot plant (*Daucus carota*)

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ABSTRACT: The aim of study was to determine the effect of lactic acid bacteria inoculants on the quality and chemical composition of waste carrot plant silage (*Daucus carota*). The study is expected to be useful as a guide and information about making a carrot plant silage waste effectively and efficiently. Using experimental methods, nested completely randomized design, with treatments of different types of bacteria: *Lactobacillus collinoides, Lactobacillus delbrueckii* and the mixture (*Lactobacillus collinoides* and *Lactobacillus delbrueckii* 1:1), and the treatment time of incubation: 2 days, 3 days, 5 days 10 days 15 days and 21 days to factor nested types of bacteria. Each treatment was repeated 3 times. The results showed that lactic acid bacteria inoculants effect ($P < 0.05$) on the quality and rate of change of chemical composition on carrot plant waste silage. Inoculants *Lactobacillus delbrueckii* bacteria was significantly the most effective way to condition an ensilase process at low temperature, low pH, low butyric acid content and high lactic acid content, compared to other types of inoculants. Silage carrots plants were added *Lactobacillus collinoides , Lactobacillus delbrueckii* and mixtures, obtained an average temperature of 26,83, 25,05, 25,88 ^{0}C, pH 3.71, 3.50, 3.34, 3,45 lactic acid content 10,10 %, 12.67% and 11,17% and butyric acid content of 0.65%, 0.30%, 0.38%. Silage that uses bacterial inoculants *Lactobacillus delbrueckii* was able to treat a small decrease in the level of the chemical composition of carrots for ensilage waste, other than another inoculants. The average reduction in chemical composition during silage ensilase using *Lactobacillus collinoides, Lactobacillus delbrueckii* and mix, dry matter content 34,61%, 37,36% and 35,64%, organic matter (% DM) 87,74, 88,44 and 88,14 crude protein (% DM) 7,72, 8,33 and 7,97. Neutyral Detergent Fiber (% DM) : 41,86, 48,10 and 45,82. Acid Detergent Fiber (% DM) : 30,05, 31,40 and 31,61 and Cellulose (% DM) 23,72, 25,64, 24,96. In addition, the inoculants *Lactobacillus delbrueckii* was also able to accelerate the stagnation and deterioration rate of change of chemical composition, ie, during five days, compared to other inoculants for 10 days. Recommended procurement of waste silage good carrot plant, using inocula of *Lactobacillus delbrueckii*.

Key words: silage, waste carrot plant, lactic acid bacteria

INTRODUCTION

Waste of carrot plant (*Daucus carota*) is a material commonly discarded because the process of thinning plant farmers. Thinning deliberately done for the purpose of reducing competition utilization of soil nutrients, and economically is intended to enhance plant productivity. Carrot plant waste such as leaves, plant stalks and stems. According Muwakhid, (2009), the amount of waste discarded carrot plants reach 7 tons per hectare per plantping period. Carrot waste obtained in fresh condition at the same time and in quantity, so that the carrot plant waste should be stored in the form of silage. The aim of this study is to determine the effect of lactic acid bacteria inoculants type and duration of incubation on the quality and chemical composition of waste of silage carrots plant. It is expected to be useful as information about waste silage making good carrot plant.

MATERIALS AND METHODS

Carrot plant waste that used is the whole part of the existing waste. The sample was taken from the carrot garden in Sumber Brantas village Bumiaji sub district Batu City . Inoculants of lactic acid bacteria is *Lactobacillus delbrueckii* and *Lactobacillus collinoides* is the result of selection in bacteria indigenous vegetable waste (Muwakhid, 2005). Inoculants was applied to 10^6 cfu g^{-1} fresh weight (Ohshima et al., 1997) and added molasses 4% fresh ingredients (Ohmomo et al., 2002).

Research was done by experimental methods, nested completely randomized design, with treatments kinds inoculants *Lactobacillus collinoides*, *Lactobacillus delbrueckii*, mixed (a combination of *Lactobacillus collinoides, Lactobacillus delbrueckii* 1:1), and the treatment time of incubation 2 days, 3 days, 5 days 10 days , 15 days and 21 days nested within factors of bacterial species. The effect of length of incubation range and effect of inoculants in a kinds of inoculants for the analysis of variance and treatment effect followed by least significant difference test.

Parameters that observed in the form of silage temperature, pH silage, lactic acid content and butyric acid content of silage, and dry matter content (DM), organic matter (OM) and crude protein (CP) silage. Electric pH meter used for pH determination of silage and silage extraction performed according to instructions (Nahm, 1992). The extract was injected on the gas as much as 0.5 µlt chromatografi using FFAP column (HP) at a temperature of 60-230 ^{0}C, the standard use of lactic acid, butyric acid and 96 per cent. Dry matter was determined by oven drying at 60 ^{0}C and 105 0C. Ash was obtained through a heating furnace 600 0C, and crude protein determined by Kjeldahl method (AOAC, 1980). The measurement NDF, ADF and cellulose (Goering and Van Soest 1970). Each measurement was conducted in duplo.

RESULTS AND DISCUSSION

Results showed that differences in types of inoculants caused significant (P <0.01) on pH, lactic acid and butyric acid, as well as significant (P <0.05) on temperature. Inoculants *Lactobacillus delbrueckii* was proven to control the pH, temperature and lowest content of butyric acid, contrary to spur the highest lactic acid content compared with inoculants *Lactobacillus collinoides* and inoculants mixtures (Table 1).

Table 1. Effect of inoculants on all kinds of old incubation on average, pH, temperature, lactic acid, and butyric acid silage

	Parameter			
Action	Temperature (^{0}C)	pH	Lactic Acid (g/kg)	Butyric Acid (g/kg)
Lactobacillus collinoides	53^b	3,50^C	10,10^A	0,65^C
Lactobacillus delbrueckii	25,05^a	3,34^A	12,67^C	0,30^A
Compound	25,88^a	3,45^B	11,17^B	0,38^B

A-C Different superscript in the same column showed highly differences (P <0.01)
a - c Different superscript in the same column indicate differences (P <0.05).

The high content of lactic acid in silage using inoculants *Lactobacillus delbrueckii* caused by the high number of bacterial cells of *Lactobacillus delbrueckii* that is was able to grow due to its ability to adapt better to the carrot plant waste. Results of the research of Muwakhid (2005), states that *Lactobacillus delbrueckii* is able to adapt to the media market vegetable waste is better than with *Lactobacillus collinoides*. The higher population of *Lactobacillus delbrueckii* will be able to form more complex enzyme. Axelsson (1998) explains that the enzyme complex have a role during the formation of lactic acid is glucokinase, fruktose-1 0.6-diphosphat aldolases, gliceraldehid -3 - phosphate dehydrogenase, and lactat Pyruvat dehydrogenase kinase. These enzymes can convert one mole of glucose into two moles of lactic acid and every one mole of fructose can be converted into two moles of lactic acid. So that a high population of lactic acid bacteria, will be able to optimize the formation of lactic acid in the silo environment (Filya, 2000). The effect is accumulation of lactic acid would be higher.

Silage pH that used inoculums *Lactobacillus collinoides*, *Lactobacillus delbrueckii* and inoculums mixture under pH 4, pH condition was sufficient to support the process ensilase, for good silage can occur if the pH of silage have been able to achieve less than 4.5 (Ohshima, et al., 1997). Low pH silage during the experiment was caused by high lactic acid formed during the process ensilase progress. Accumulation of lactic acid will show in the decrease in silage pH values (Henderson, 1993).

pH conditions which should decline in the use of silage inoculants *Lactobacillus delbrueckii* can inhibit the activity of Clostridium species in the silo. Whereas dekomposis activities for Clostridium

species utilize carbon and energy converts into heat (Ohmomo et al., 2002). Thus the addition of inoculants *Lactobacillus delbrueckii* was able to suppress the lowest temperature, compared with temperatures in silage using inoculants *Lactobacillus collinoides* and inoculants mixture.

Clostridium species was able to convert lactic acid into butyric acid during process of ensilase (Filya, 2000), a Silage using inoculants *Lactobacillus delbrueckii* can reduce the activity of Clostridium species better than the use of silage inoculated with *Lactobacillus collinoides* and inoculants mixture, resulting in the butyric acid content of silage using inoculants *Lactobacillus delbrueckii* lowest compared with the use of silage inoculants and inoculants *Lactobacillus collinoides* mixture.

Long incubation caused significant (P <0.01) against temperature, pH, lactic acid, and butyric acid silage, the inoculants of *Lactobacillus collinoides*, *Lactobacillus delbrueckii* and inoculants mixture. Figure 1 shows that silage using inoculants *Lactobacillus collinoides* and inoculants mixture temperature was increased along with increasing time of incubation, until the time of incubation 10 days,.

Whereas silage using inoculums *Lactobacillus collinoides* and inoculums mixture pH decreased along with increasing time of incubation, until the time of incubation 10 days, and then stagnated for a long incubation period of 21 days. While silage using inoculums *Lactobacillus delbrueckii* decreased the pH up to 5 days and then stagnated for a long decline in 21 days incubation. Use of inoculums *Lactobacillus delbrueckii* had reached pH 3.6 on day 5, this is achieved immediately capable of controlling the stability of the anaerobic degradation of silage (Ranjit and Kung, 2000).

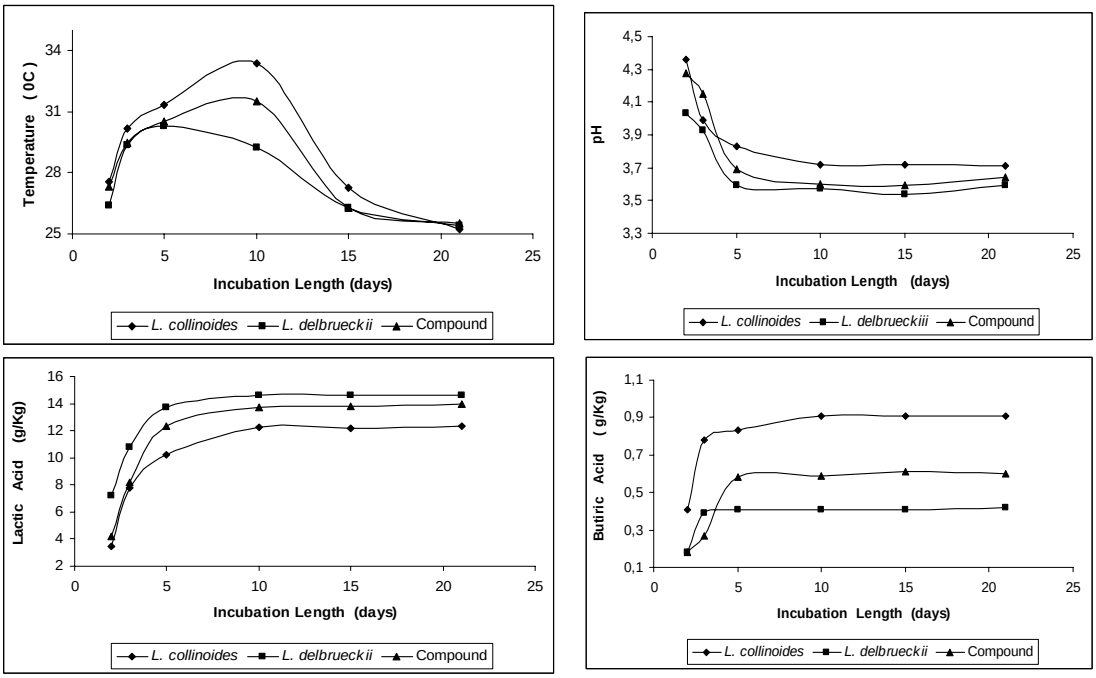

Figure 1. Temperature and pH conditions, lacticic acid and Butiric acid content on the Old Incubation Berbea Silage

Figure 1 explains *Lactobacillus delbrueckii* was obtained actic acid content at the time of stagnation, compared with the silage using inoculants *Lactobacillus collinoides* and inoculants mixture. This occurs because lactic acid is formed from a soluble material carbohidrat, through enzymatic processes by an enzyme complex that is formed by lactic acid bacteria (Salminen et al., 1998). Silo environment dominated by lactic acid bacteria will be fulfilled the target optimization of enzymatic reactions in the formation of lactic acid (Muck, 2002). Added incubation time can ensure the growth of lactic acid bacteria population, along the pH conditions still allow for microbial growth

in the silo (Ohshima et al., 1997) If the conditions in the silo pH less than 4, the activity of lactic acid bacteria from obstructed (Mc. Donald, 1991), so that lactic acid formation process becomes unstable.

The duration of incubation resulted in increased butyric acid content, until at a certain time of stagnation. Silage using inoculums *Lactobacillus collinoides* start stagnation in 10 days of incubation and inoculums mixture in the long stagnation began five days of incubation, whereas the use of silage inoculums *Lactobacillus delbrueckii* on long stagnation began three days of incubation.

Butyric acid is formed from the conversion of lactic acid into butyric acid, CO_2 and H2 from the role of bacteria that are still active (Ohmomo et al., 2002). At pH less than 4, the decline in the quality of silage to be stable during the stay under anaerobic conditions (Henderson, 1993). Conversely, if oxygen or water supply occurs in the silo, the pH is increased and the fermentation of clostridium bacteria can take place, on condition it may also break down the lactic acid into butyric acid (Ohmomo et al., 2002).

Inoculants significant difference ($P < 0.05$) against the content of DM, OM, CP, NDF, ADF and cellulose Silage. Table 2 states that differences in content of DM, OM, CP, NDF, ADF and cellulose in silage using inoculants *Lactobacillus delbrecki* caused by the limited activity of microbial spoilage. Microbial spoilage, such as Bacterium herbicola, Escherichia coli, Bacillus cereuis, Listeria monocytogenes can break down organic materials and proteins into CO2, CH4, CO, NO, NO_2 and water (Ohmomo et al., 2002). Lactic acid bacteria can produce hydrogen peroxide, will inhibit the growth of microbial spoilage.

Table 2. How the influence of inoculants in all the Old Incubation Rata-rata on dry material content (DM), Organic Materials (OM), Crude Protein (CP) Silase

	Parameter					
Action	DM (%)	OM (% BK)	CP (% BK)	DF (% BK)	ADF (% BK)	Cellulose (% BK)
Inokulum L. collinoides	34,61a	87,74[a]	7,72[a]	41,86[a]	30,05 [a]	23,72[a]
Lactobacillus delbrueckii	37,36[c]	88,44[b]	8,33[c]	48,10[c]	31,40 [b]	25,64[c]
Compound	35,64[b]	88,14[b]	7,97[b]	45,82[b]	31,61[b]	24,96[b]

Different superscript in the same column indicate significant differences ($P < 0.05$).

Hydrogen peroxide inhibits the growth of microbial spoilage in various ways, including through the influence of oxidation on microbial cells (Brashers et al., 1998), or can be through solving the basic structure of the nucleic acid molecule or protein, cells (Jin et al., 1996). Activities of hydrogen peroxide as an anti-microbial compounds, allegedly involving the lactoperoxidase system. This system may damage the cytoplasmic membrane of Gram-negative bacteria, as Gram-positive bacteria group, has a cell membrane is more able to fortify the action of lactoperoxidase compared with the group of Gram-negative bacteria (VanDevoorde et al., 1994). Ensilase process is good, it will immediately stop the reform of plant cells by *Listeria monocytogenes*. According Ballongue (1993), during the ensilase process, lactic acid bacteria have a role in decreasing pH of silage in the silo to below 4.2. At low pH conditions of Listeria monocytogenes incapable of survival. Because the ideal conditions for the life of *Listeria monocytogenes* approximately 5.7 to 8.9. (Mc. Donald, 1991).

Ensilase process was dominated by a high population of lactic acid bacteria, that can immediately stop the activity of microbial spoilage (Cai et al., 1999). The results of Meeske et al. (1999), also proves that *Digitaria eriantha* silage using mixed inoculums of *Lactobacillus plantarum, Streptococcus faecium* and *Pediococcus acidilactic*, obtained by the content of DM, OM and CP respectively 43.2%, 91.7% and 6.1% higher than with no addition of inoculums by 38.2%, 90.5% and 5.9%.

Long incubation in silage using inoculants *Lactobacillus collinoides* significant ($P < 0.05$) against the average content of DM, OM, CP, NDF, ADF and Cellulose but significant ($P < 0.01$) in silage using inoculants *Lactobacillus delbrueckii* and inoculants mixture . Figure 2. explained that the increased time of incubation resulted in decreased content of DM, OM, CP, NDF, ADF silage, until at a certain time of stagnation. Silage using inoculants *Lactobacillus delbrueckii* and inoculants mixture decreased the content of DM, OM, CP, NDF, ADF and Cellulose, and stagnant decline began long incubation 5 days, this occurs faster than the stagnation of silage using inoculants *Lactobacillus collinoides*, which began a long incubation period of stagnation at 10 days.

Fresh silage materials still continue the process of respiration. Oxygen inside the silo was utilized by spoilage bacteria to grow by using the cell content of feedstuffs as sources of the medium, so that the nutrients in feed dry matter was split into CO_2, CH_4, CO, NO, NO_2 and water (Ohmomo et al., 2002). This condition is when the lactic acid bacteria in the silo develop properly, will soon be formed lactic acid to lower the pH of silage, silage pH below 4 would slow down the growth of clostridium bacteria, so the process of solving chemical components can be terminated immediately (Knicky, 2005).

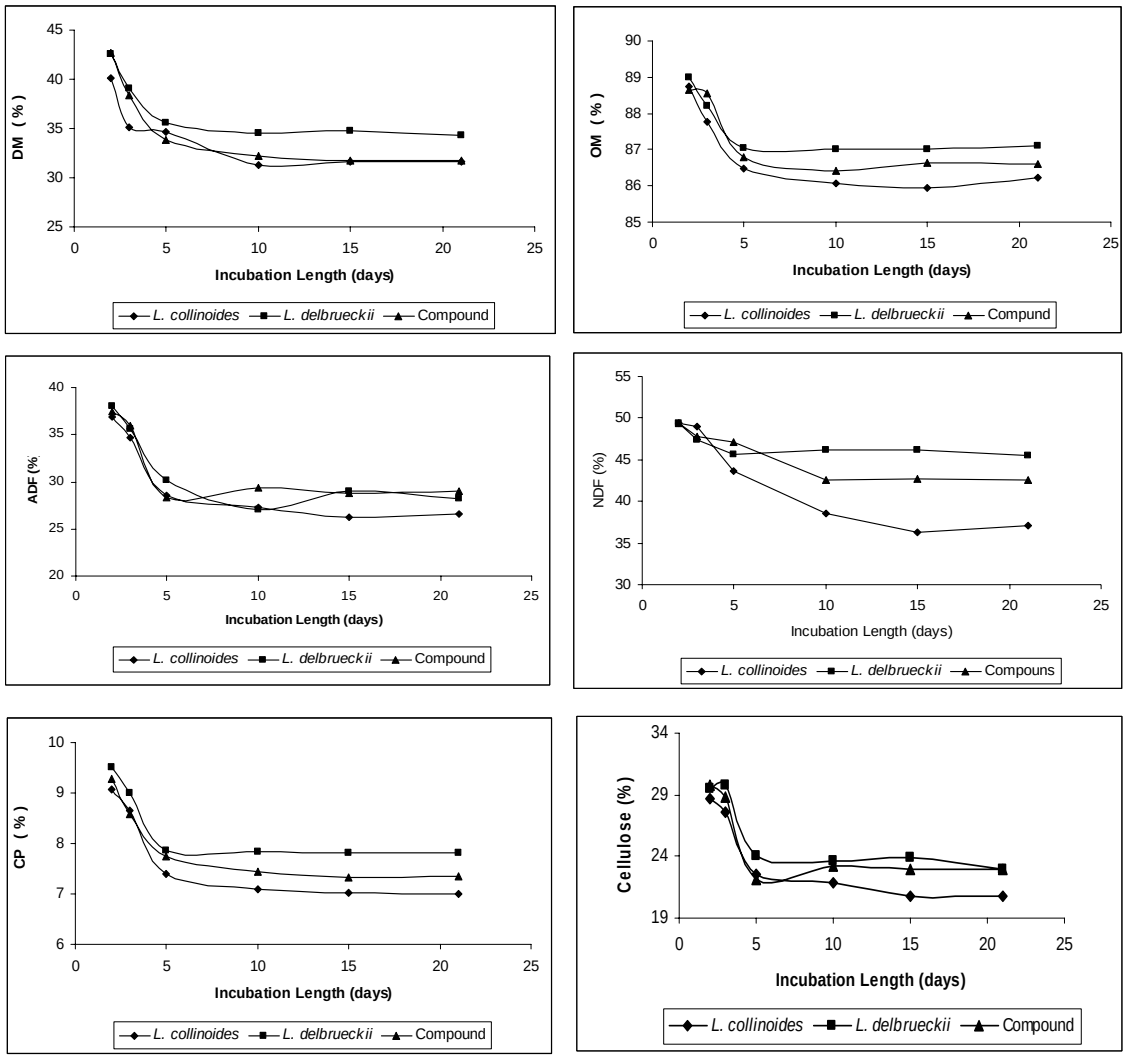

Figure 2. Dry material, organic materials , crude protein neutral detergent fiber, acit getergent fiber and cellulose silage on various time of incubation

CONCLUSIONS

The Conclusion was that the performance of ensilase carrot plant wastes could be optimally supported by the inoculants *Lactobacillus collinoides, Lactobacillus delbrueckii* and inoculants mixture. Inoculants *Lactobacillus delbrueckii* most effective way to condition a silage at low pH, low temperature low butyric acid content and high lactic acid content. And also, it is effective to inhibit the decrease in content of DM, OM and CP silage. As an alternative to obtain was silage of good carrot plant wastes are advised to use inoculants *Lactobacillus delbrueckii*.

LITERATURE CITED

AOAC. 1980. Official Methoda of Analysis, 13th Edition. Association of Official Aanalytical Chemists. Washington DC.

Axelsson, L. 1998. Lactic Acid Bacteria Classification and Physiology. In Lactic Acid Bacteria. Microbiology and Functional Aspects. Salminen, S and A.V Wright (Eds). Scond Edition Revised and Expanded. Marcel Dekker, Inc. New York. pp 1 - 58

Ballongue, J. 1993. Bifido Bacteria and Probiotic Action. In Lactic Acid Bacteria Micribiology and Functional Aspects. Salminen, S and A.V. Wright (Eds). Marcel Dekker Inc. New York. pp 245 - 249

Brashears, M.M., S.S. Reilly, and S.E. Gilliland. 1998. Antagonistic of Cells of Lactobacillus lactis Toword Escherichia coli 0157 : H7 on Revigerated Raw Chicken Meat. J. Food Protect. 61 : 166 - 170

Cai, Y., Y. Benno, M. Ogawa and S. Kuma. 1999. Effect of Applying Lactic Acid Bacteria Isolated from Forage Crops on Fermentation Characteristics and Aerobic Deterioration of Silage. J. Dairy Sci. 82 : 520 – 526

Goering, H.K., and Van Soest. 1970. Forage Fiber Analysis (Apparatus, Reagents, Procedures and Some Applications). Agricultural Research Service. United States Departement of Agriculture. Washington D.C

Henderson, N. 1993. Silage Additives. J. Anim. Feed Sci. and Tecnol 45 : 35 – 56

Filya, I. G. Ashbell, Y. Hen and Z.G. Weinberg. 2000. The Effect of Bacterial Inoculants on the Fermentation and Aerobic Stability of Whole Crop Wheat Silage. Anim. Feed Sci. and Tecnol. 88 : 39 – 46

Jin, L.Z., N. Abdullah, M.A Ali and S. Jalaluddin. 1996. Antagonistic Effects of Intestinal Lactobacillus Isolates on Phatogens of Chicken. J. Appl. Microbiol. 23 : 67 - 71

Knicky, M. 2005. Posibilities to Improve Silaage Conservation. Effects of Crop, Ensiling Technology and additives. Doctoral Thesis. Swedish University of Agricultural Sciences Uppsala. 2005

McDonald, P., N. Henderson, and S. Heron. 1991. The Biochemistry of Silage. Chalcombe Publications. Marlow.

Meeske, R., H.M. Basson and C.W. Cruywagen. 1999. The Effect of a Lactic Acid Bacterial Inoculant With Enzymes on the Fermentation Dinamics, Intake and Digestibility of Digitaria eriantha silage. J. Anim. Feed Sci. and Tecnol. 81 : 237 – 248

Muwakhid, B. 2005. Isolasi, Seleksi dan Identifikasi Bakteri Asam Laktat isolat sampah Organik Pasar. Disertasi Doktor. Program Pasca sarjana Universitas Brawijaya. Malang

Muck, R.E. 2002. Effects of Con Silage Inoculants on Aerobic Stability. An Asae Meeting Presentation. The Society In Agricultural, Food and Biological Systems. Chicago. July 28 – 31, 2003

Muwakhid, B. 2009. Potensi limbah tanaman Wortel sebagai Pakan Ternak Ruminansia. J. Al Buhuts. Exact. (7) 156 - 160 .

Nahm, K.H. 1992. Practical guide to feed, forage and water analisys. Yoo Han Publ. Seoul.

Ohmomo, S., O. Tanaka, H.K. Kitamoto and Y. Cai. 2002. Silage and Microbial Performance, Old Story but New Problems. J. JARQ 36 (2) 59 – 71

Ohshima, M., E. Kimura, H. Yokota. 1997. A Method of Making Good Quality Silage From Direct Cut Alfalfa by Spraying Previously Fermented Juice. J. Anim. Feed. Sci. Technol. 66 : 129 - 137

Ranjit, N.K. and L. Kung. 2000. The Effect of Lactobacillus buchneri, Lactobacillus plantarum, or A Chemical Preservative on The Fermentation and Aerobic Stability of Corn Silage. J. Dairy Sci. 83 : 526 – 535

Salminen, S. And AS. Wright. 1998. Lactic Acid Bacteria. Microbiology and Functional Aspects. Scont Adition. Marcel Dekker, Inc. New York

VanDervoorde, L., VanDewoestyne, B. Bruyneel, H. Christiaeus and W. Verstraete. 1994. Critical Factor Governing the Competive Behaveor of Lactic Acid Bacteria in Mixed Cultures. In the Lactic Acid Bacteria. Volume I. The Lactic Acid Bacteria in Health and Disease. Brian, J and N.V. Wood. (Eds). Lactic Academic and Proffessional. London. pp 356 - 367