The role of lactic acid bacteria on silage duration process and rumen content silage quality

Isnandar,* Ristianto Utomo,† Siti Chuzaemi,‡ Endang Sutariningsih,§ and Lies Mira Yusiati†

*****PhD Student of Animal Science Faculty Gadjah Mada University Yogyakarta Indonesia (staff of Animal Husbandry Training Center for Batu); †Faculty of Animal Science Gadjah Mada University Yogyakarta Indonesia; ‡Faculty of Animal Science Brawijaya University, Malang Indonesia; and §Faculty of Biology Science Gadjah Mada University Yogyakarta Indonesia

ABSTRACT: This research was a continuation of previous research aimed to find out the role of three inoculum Lactic acids bacteria/LAB (*L. plantarum, L. Spp* (from rumen content), and L. mixed (*L. acidophilus, L. plantarum, L. cacei,* and *L. Spp*)) is dominant on best molasses additive levels (8%) to silage duration process and silage quality of rumen content. Rumen content of 71.43 g and dried cassava pomace 28.57 g was mixed evenly (dry matter 35%, approximately), given 0.1% three kinds of LAB (*L. plantarum, L. Spp,* and *L.* mixed), with 4 silage duration process (1, 2, 3, and 4 weeks) in ambient temperature (25^{o}C approximately), it was a 3 x 4 completely randomized design factorial with four replications. Plastic jars were used as silo. Variables were evaluated in a laboratory silage quality. Concluded that the use of inoculants *Lactobacillus plantarum* utilization and molasses 8% resulted best rumen content silage ($P<0.05$) with 2 weeks silage process duration.

Key words: lactic acid bacteria, rumen content, silage quality, duration

INTRODUCTION

Feed was the main priority factor for production (56-60%) in the livestock business, so we need to develop strategic aimed at the utilization of unconventional feed resources, such as agricultural waste, livestock waste, agricultural and industrial waste. One of the livestock waste that can be used as cattle feed is the slaughter house waste (cow rumen content), which has been discarded or used as organic fertilizer, but still had potential as a ruminant feed.

Use of rumen contents as cattle feed reported by Messermith (1973), by using rumen contents as feed materials up 15 percent could produce daily body weight gain (ADG), feed intake, efficiency, and feed conversion was not significantly different from the control diet. In terms of chemical composition of rumen contents, it contains 12.2% crude protein (CP), 25% crude fiber (CF), 5.2% ether extract (EE), ash 7.9%, and 49.6% nitrogen free extract (NFE) (Ricci, 1977; Isnandar 2001), making it suitable for ruminants feed. The main constraint as a source of feed is its odors, so the cows is refused to eat, and it's easy to decompose (Witherow and Lammers, 1976)

One way to eliminate odor, prevent spoilage and maintain nutrients is making a silage, which is preserved by fermentation. Preservation is based on a high acidity due to lactic acid formation. The more lactic acid is formed, the higher acidity in order to obtain better silage. According to Bo Gohl (1981), low pH can also suppress the growth of parasites and pathogens microorganism. These conditions can be generated when the pH of silage is less than 4.2 or the range of 3.8 to 4.2 (Church, 1986).

Making silage additives are often added ingredients such as rice bran, corn bran, cassava, and molasses to increase the content of dry matter and as a source of soluble carbohydrate could stimulate fermentation. Addition of lactic acid bacteria inoculants to the silage process aimed to obtain faster and improve the quality of silage. Lactic acid bacteria (BAL) is one of the bacteria contained in the rumen content (Jouany, 1991), but still required an assessment of its ability to produce lactic acid in the process of making silage from the rumen content.

Cassava pomace (*onggok* or *gamblong*) is a solid residue of cassava processing byproduct into leveling compound (cassava starch). Processing of cassava into cassava starch produce 15% to 20% cassava peel and 5% to 20% cassava pomace (Judoamidjojo *et al.,* 1989). Molasses is a highly viscous

liquid and brown color from the rest of the sap which has undergone a process of purification, concentration and intake of sugar through the process of crystallization (Tedjowahjono, 1987). Making Bermuda grass silage cut with a dry matter (DM) 32.4% and neutral detergent fibers (NDF) 70.2% which were treated using molasses (DM 97%), with four levels (0, 4, 8, and 12%) were given inoculation of 1.7 l /tone of fresh material, give results of increasing levels of molasses lower the pH, acid detergent fiber (ADF) and NDF, increased digestibility of dry matter in vitro (Nayigihugu *et al.*, 1995).

This study is a continuation of first research to find out the role of the three inoculums of lactic acid bacteria (*Lactobacilus. plantarum, L. Spp* (rumen content), and a mixture of *L.* (*L. acidophilus, L. plantarum, L. cacei, and L. Spp*)) dominant at the level of best molasses additive (8%) against the ensiles duration and silage quality of rumen content.

MATERIALS AND METHODS

Research materials were consisted of three different inoculants: *Lactobacillus plantarum, Lactobacillus Spp* isolated from the rumen, and a mixture of *Lactobacillus sp* (*L. acidophilus, L. plantarum, L. cacei, and L. Spp*). Rumen contents obtained from the slaughter house in Malang City. Cassava waste obtained from cassava starch factory in the Kandangan District – Kediri Regency. Molasses is sugar factory waste obtained from the Kebunagung sugar factory in Malang Regency.

Equipment used was a set of tools testing lactic acid, Acetic acid, butyric acid at the Laboratory of Nutritional Biochemistry Faculty of Animal Science, Gadjah Mada University (UGM), testing of ammonia (NH_3-N) Inter-University Laboratory of Biochemistry UGM Yogyakarta, determining the chemical composition of feed done at the Laboratory of Animal Nutrition Animal Science, Training Center for Batu, and Animal Nutrition Laboratory of Beef Cattle Research Institute, Grati, Pasuruan, Indonesia.

The study was conducted using completely randomized design (CRD) factorial consisting of 2 factors (Steel and Torrie, 1991). The first factor consisted of three inoculants: *Lactobacillus plantarum, Lactobacillus sp.* from the rumen content, and *Lactobacillus sp.* mixed. The second factor consisted of four levels ensilage duration, namely 1, 2, 3, and 4 weeks, each performed four repetitions. Each unit of the experiment was done using rumen content 1428.60 g, cassava pomace 571.40 g, and molasses 8%, after treated with 0.1% *Lactobacillus* bacterial inoculants. Evenly mixed, put into plastic jar capacity 2 kg, closed tight, stored at room temperature of about 25^{o}C. Silage harvested after ensilage lasted 1, 2, 3, and 4 weeks.

Variables measured were pH silage (Nahm, 1992), the content of lactic acid, acetic acid, and butyric acid (AOAC, 1980), N-NH_3 (Alexander *et al*., 1985), dry matter, organic matter, crude protein, crude fiber, and nitrogen free extracted (Harris, 1970).

RESULTS AND DISCUSION

Circumstances before Ensilage

The materials used to make silage were a mixture of rumen content, cassava pomace, and molasses. Chemical composition of silage made of those materials was listed in Table 1. Table 1 showed that OM and CP content of the rumen was 85.34% and 11.23% respectively, it was higher than cassava pomace. Dry matter cassava pomace was (85.12%), higher than the DM of rumen content (15.42%). Furthermore, 71.43% of rumen contents and 28.57% of cassavas cassava pomace can produce about 35% dry matter, added molasses as much as 8% and 0.1% of *Lactobacillus* bacterial inoculants. The result of proximate analysis performed at the Laboratory of Nutrition Loka Sapi Potong Grati-Pasuruan and Nutrition Laboratory Animal Husbandry Training Center Batu, shows DM (35.56%), OM (81.25%), CP (7.03%) CF (19.99%), and NFE (64.14%). This situation indicated that in addition gave the inoculants is also observed from the dry matter content and nitrogen free extract made eligible to make silage.

Table 1. Chemical composition of materials used in manufacture of silage (% / BK)

Component	Rumen content	Cassava pomace	Molasses
DM	15.42	85.12	66 - 71
OM	85.34	80.11	78.21
CP	11.23	0.90	3.40
CF	22.19	16.49	18.11
NFE	51.56	60.47	54.45
Water Soluble Carbohydrate	undetection	12.51	43.11

Source : Nutrition Laboratory analysis Loka Sapi Potong Grati-Pasuruan and Nutrition Laboratory Animal Husbandry Training Center for Batu

Circumstances after Ensilage

The observation that the quality of silage made through laboratory tests, are presented in Table 2.

Table 2. Average pH, lactic acid content, acetic acid, butyric acid, N-NH3 silage (% DM)

Treatment	pH	Lactic acid	Acetic acid	Butyric acid	N-NH_3
Inoculants					
L plantarum	4.07 ^{a}	4.70 ^{c}	0.45^{a}	0.05^{a}	1.85 ^{a}
L.campuran	4.13 ^{a}	3.88 ^{b}	0.71^{b}	0.07^{b}	2.08 ^{b}
L.spp rumen content	4.44 ^{b}	3.46 ^{a}	0.84^{c}	0.10^{c}	2.42 ^{c}
Duration					
Week I	4.74 ^{w}	0.58 ^{w}	0.24^{w}	0.01^{w}	0.03 ^{w}
Week II	4.19 ^{x}	4.65 ^{x}	0.80^{x}	0.10^{x}	2.37 ^{x}
Week III	3.98 ^{y}	5.38 ^{y}	0.81^{x}	0.10^{x}	2.71 ^{xy}
Week IV	3.95 ^{y}	5.43 ^{y}	0.81^{x}	0.10^{x}	3.04 ^{y}
Interaction					
L plantarum week I	4.38^{o}	0.66 ^{l}	0.21^{k}	0.01^{k}	0.03^{k}
L plantarum week II	4.03^{mn}	5.19 ^{q}	0.53^{m}	0.06^{m}	2.11^{l}
L plantarum week III	3.95^{l}	6.47 ^{r}	0.53^{m}	0.07^{n}	2.11^{l}
L plantarum week IV	3.93^{k}	6.48 ^{r}	0.53^{m}	0.07^{n}	2.11^{l}
L mixed week I	4.43^{p}	0.60 ^{l}	0.21^{k}	0.01^{k}	0.02^{k}
L mixed week II	4.25^{no}	4.76 ^{o}	0.87^{n}	0.09^{o}	2.54^{n}
L mixed week III	3.93^{k}	5.06 ^{p}	0.88^{n}	0.09^{o}	2.54^{n}
L mixed week IV	3.94^{k}	5.08 ^{p}	0.88^{n}	0.09^{o}	3.55^{p}
L spp rumen conten week I	5.43^{q}	0.49 ^{k}	0.30^{l}	0.02^{l}	0.04^{k}
L spp rumen conten week II	4.29^{no}	4.01 ^{m}	1.01^{o}	0.13^{p}	2.46^{m}
L spp rumen conten week III	4.08^{mn}	4.62 ^{n}	1.02^{o}	0.13^{p}	3.47^{o}
L spp rumen conten week IV	3.96^{lm}	4.72 ^{o}	1.02^{o}	0.13^{p}	3.47^{o}

^{a,b,c} : Different superscript in the same column indicate differences (P <0.05) treatment of the use of inoculants

$^{w, x, y, z}$ Different superscript in the same column indicate differences (P <0.05) treatment ensilage duration

$^{k - v}$ Different superscript in the same column indicate differences (P <0.05) interaction between treatment with the use of inoculants ensilage time

Lactic Acid Content and pH of Silage. The results (Table 2) showed that use of inoculants treatment was significantly (P <0.05) affect the silage pH and lactic acid content. Use of *L plantarum* produce higher lactic acid (4.70%) and lower pH (4.07) than the use of *L* mixed *spp* and *L spp* from rumen contents containing lactic acid 3.88% and 3.46%, pH 4.13 and 4.4, respectively. Quality silage can be said good, because according Belirem and Ulpesli (1960) in the McIlroy (1977), the good

quality silage have 1.5 to 2.5% lactic acid content and pH lower than 4.2. This is due to the ability of lactic acid bacteria in rumen contents of silage fermentation process is varied, in line with the opinion of Muck (2004) who stated that the fermentation ability of each species of Lactobacillus is not the same. According to Rodriguez *et al.* (1994) using *L. plantarum* inoculants alone or mixed on improving the quality of silage causing lower pH and higher lactic acid content than without inoculants. Lactic acid bacteria are generally able to grow at a temperature of 15^{o}C, do not grow at a temperature of 45^{o}C (McDonald, *et al*., 1991). This temperature is in line with the room temperature where the silo was stored.

Ensilage duration significantly affecting (P <0.05) pH and lactic acid content of silage. At the first week ensilage has been slow lactic acid fermentation, with 4.74 pH and 0.58% lactic acid content was lower than the second week of 4.65% lactic acid. Lactic acid fermentation process occurs most rapidly in second week with 4.19 pH and 4.65% lactic acid content. The third weeks has been slow lactic acid fermentation begins, with lactic acid content of 5.38% and the fourth week of lactic acid fermentation was slow (start stop) lactic acid content of only 5.43%. Lactic acid content at the fourth week was not significantly different compared to third weeks. Trends in the rate of fermentation under the direction of the long ensilage Harris (2003), that the process ensilage until achieving the formation of lactic acid and pH between 3.5 to 4.2 are at days 15-20, after it reached a stable condition so that the silage to be durable.

The interaction was significantly (P <0.05) affect on pH and lactic acid content of silage. *L plantarum* inoculants treatment was produces lactic acid content of 0.66, 5.19, 6.47, and 6.48% at the first week, second week, third week, and fourth week are higher than L mixed spp inoculants consecutive 0 , 60, 4.76, 5.06, and 5.08% and L spp from rumen contents, respectively 0.49, 4.01, 4.62, and 4.72*%. L plantarum* inoculants treatment was produce silage with 4.38, 4.03, 3.95 and 3.93 pH at the week, the second, the third and the fourth week to significantly (P <0.05) lower than the use of L mixed inoculants successively 4.43, 4.25, 3.93 and 3.94 as well as L spp rumen contents were successively 5.43, 4.29, 4.08 and 3.96. At the second week all inoculants treatments had reached a high lactic acid production and meet the minimum production of lactic acid content of 1.5 to 2.5% of good quality silage.

The Content of Acetic Acid, Butyric Acid, N-NH$_3$. The results (Table 2) showed that the use of inoculants effect was significantly (P <0.05) to the content of acetic acid, butyric acid and N-NH$_3$. The silage by *L plantarum* inoculants treatment was containing 0.45% acetic acids, butyric acids 0.05%, N-NH31, 85% lower than the use of L spp mixed inoculants of and L spp rumen content, in chronological succession of acetic acid containing 0.71 % and 0.84%, 0.07% butyric acid and 0.10%, N-NH$_3$ 2.08% and 2.42%. Lactic acid content and pH of silage fermentation processes determining the formation of acetic acid, butyric acid, protein degradation, and formation of ammonia by clostridia bacteria. The faster the fermentation of lactic acid and the decline in silage pH can immediately stop the clostridia fermentation. Use of *Lactobacillus plantarum* inoculants LAB homo fermentative able to increase the population, increasing lactic acid fermentation, accelerating the decline in silage pH thus suppress protein degradation into the N-NH$_3$ (Shaver, 2008). In line with Meeske *et al*. (1993) that the use *L. plantarum* inoculants of silage, N-NH$_3$ content of silage produced 0.9 grams smaller than without using the inoculation of 1.7 g / kg of N total. The good quality silage has a content ratio of acetic acid and lactic acid 1: 3 (Kung, 2001).

Ensilage duration significantly affect (P <0.05) on the content of acetic acid, butyric acid, N-NH$_3$. The achievement of high lactic acid content and low pH followed by a steady depletion of fermentation by clostridia which produce acetic acid, butyric acid, protein degradation and the formation of N-NH$_3$. Acetic acid and butyric acid in the silage caused fermentation by clostridia, less rapid fermentation of lactic acid, and pH is still high (Muck, 1987).

There was significant interaction (P <0.05) between the use of inoculants with ensilage duration of acetic acid, butyric acid, and N-NH$_3$ silage. Acetic acid fermentation occurred in the early ensilage 24-72 hours (Schroeder, 2004). High water content stimulates proteolytic activity that produces a high pH from pH 4.0 to 4.8 (Van Saun and Heinrichs, 2008).

Dry Matter, Organic Matter, Crude Protein, and Nitrogen Free Extract Decreased, and Crude Fiber Increased

Decrease of DM, OM, CP, and NFE , and decreased of CF increased were shown in Table 3. The DM, OM, CP, NFE, and CF content analysis after a silage than before in making silage showed that use of inoculants and ensilage duration has significantly effect (P <0.05) toward a decrease of DM, OM, CP, NFE and increased of CF. The smallest decrease of DM, OM, CP, and CF content, and NFE increase was of the *L plantarum* inoculants treatment, followed by *L spp* mixed, and *L spp* rumen content. Trend of reduced rates of DM, OM, NFE and increased the percentage of CF showed that a decrease in DM, OM, CP, NFE and increase in CF most rapidly increase occurred during the first week and the second week ensilage compared to the third and fourth week. Increasingly lower DM content of the downward trend in third and the fourth week, since the low pH and the more slowing silage fermentation process. Table 2 showed that at the second week using *L plantarum* had reached 4.03 pH as the pH is ideal to be achieved, followed by *L spp* mixed and *L spp* rumen content at the third week. According to Harris (2003), the fermentation process begins 3-5 days after filling up to 15-20 days of ensilage, during the fermentation of lactic acid produced to the highest pH reached 3.8 to 4.2.

Table 3. Decrease in DM, OM, CP, NFE (%) and increased CF (%) silage

Treatnment	DM	OM	CP	NFE	CF
Inoculants					
L plantarum	3.76^{a}	4.49^{a}	2.45^{a}	3.37^{a}	1.69^{a}
L.campuran	4.37^{b}	5.00^{a}	2.61^{b}	3.46^{b}	2.15^{a}
L.spp rumen content	4.37^{b}	5.04^{a}	2.79^{c}	3.65^{c}	4.44^{b}
Duration					
Week I	2.12^{w}	2.22^{w}	0.56^{w}	1.24^{w}	0.57^{w}
Week II	4.14^{x}	5.43^{X}	3.19^{x}	4.17^{x}	2.47^{x}
Week III	5.14^{y}	5.78^{X}	3.30^{xy}	4.27^{y}	3.81^{x}
Week IV	5.26^{z}	5.94^{Xy}	3.41^{xy}	4.29^{y}	4.17^{x}
Interaction					
L plantarum week I	2.01^{k}	2.16^{k}	0.31^{k}	1.06^{k}	0.34^{K}
L plantarum week II	4.26^{mn}	5.13^{l}	3.05^{n}	4.02^{m}	2.04^{m}
L plantarum week III	4.35^{mn}	5.21^{lm}	3.20^{no}	4.20^{no}	2.18^{mn}
L plantarum week IV	4.43^{n}	5.46^{lm}	3.23^{o}	4.21^{no}	2.19^{mn}
L mixed week I	2.16^{k}	2.25^{k}	0.46^{l}	1.13^{k}	0.75^{l}
L mixed week II	4.24^{m}	5.53^{m}	3.21^{o}	4.15^{no}	2.06^{mn}
L mixed week III	5.53^{o}	6.05^{n}	3.32^{pq}	4.26^{o}	2.74^{n}
L mixed week IV	5.55^{op}	6.16^{no}	3.46^{qr}	4.30^{o}	3.04^{no}
L spp rumen content week I	2.19^{kl}	2.27^{k}	0.90^{m}	1.54^{l}	0.64^{l}
L spp rumen content week II	3.93^{l}	5.63^{m}	3.32^{p}	4.34^{o}	3.31^{no}
L spp rumen content week III	5.55^{op}	6.07^{no}	3.39^{q}	4.36^{o}	6.53^{o}
L spp rumen content week IV	5.81^{q}	6.18^{o}	3.54^{r}	4.36^{p}	7.28^{p}

$^{a, b, c}$ Different superscript in the same column indicate differences (P <0.05) treatment of the use of inoculants

$^{w, x, y, z}$ Different superscript in the same columns indicate differences (P <0.05) treatment time ensilage

$^{k, v}$ Different superscript in the same column indicate differences (P <0.05) interaction between treatment with the use of inoculants ensilage time

According to Kunkle *et al.* (2006), at the beginning of silage when the materials incorporated into the silo, there is the respiration process which produces CO_2, water, and heat, duration of respiration depended on the amount of air in the silo. There is a hetero fermentation to produce acetic acid, lactic acid and other organic acids until the pH reaches below the 5-6, it started after air in the silo down, this time takes place for 2-4 days with rapid degradation of organic matter. This process was stopped by activity of Lactobacillus homo fermentation and pH reached 3.8 to 4.2. According to Kunkle *et al*. (2006), lactic acid fermentation and lactic acid produced in the 2-3 week period of ensilage.

According to Zimmerman (2008), the results of silage analysis: pH, lactic acid, acetic acid, propionic, butyric, alcohol, N-NH_3, and the ratio of lactic acid with acetic acid can be used as indicator for the level of silage quality. According to Bolsen (2008), a good silage indicated by DM decreased about 5-15%, while for bad silage, DM decreased more than 25%, and large decrease of DM caused by the respiration process before ensilage process. In terms of percentage of DM decrease, it can be said that silage produced in this research was still considered as a good quality silage, an average decrease of DM in the first, second, third, and forth week, were 2.12; 4.14; 5.14, 5.26%, respectively. Silage quality is considered good if a substantial reduction of no more than 15% DM (Pope, 1973).

CONCLUSIONS

Inoculants were significantly affect lactic acid fermentation, accelerating the ensilage duration two weeks shorter. *Lactobacillus plantarum* was the best inoculant compared to other inoculants. Rumen content was containing good nutrient and the chemical composition did not differ significantly after ensilage. The good quality rumen content silage still needed applied study as a ruminant feed.

LITERATURE CITED

Alexander, R.R., J.M. Griffith and M.N.L. Wilkinson. 1985. Basic Biochemical Method. A Wiley Interscence Publ. John Wiley & Son., New York, Chicester, Brisbane, Toronto, Singapore.

AOAC .1980. Official Method of Analysis of the Association of Official Analytical Chemists. 13th ed. Benyamin Franklin Station, Washington D.C.

Bo Gohl. 1981. Tropical feeds. Feeds information summaries and nutritive value. FAO, Rome

Bolsen K, K. 2008. Improving Silage Quality. Ruminant Nutrition and Forage Preservation. Kansas State University Agricultural Experiment Station and Cooperative Extension Service. Kansas.

Church, D.C. 1986. Feed and Feeding. Prentice Hill A Division of Simon and Schuster., Inc. Englewood Cliffs, NY, United Stated of America.

Harris, B. Jr. 2003. Harvesting, Storing and Feeding Silage to Dairy Cattle. the Animal Science Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida.

Harris, L.E. 1970. Nutrition Research Techniques for Domestic and Wild Animals. Volume I. Animal Science. Departmen Utah State University. Logan.

Isnandar. 2001. Kajian tentang penggunaan silase isi rumen dalam ransum konsentrat Sapi Perah Peranakan Friesian Holland (PFH) yerhadap penampilan produksi susu. Tesis. Program Pasca Sarjana Universitas Brawijaya. Malang.

Jouane, J.P. 1991. Rumen Microbial Metabolism and Ruminant Digestion. Institut National De La Recherche Agronomique. 147, rue de I'Universite – 75338. Paris.

Kung, L. Jr., 2001. What The Smell From Silages Can Tell You. University of Delaware.

Kunkle W. E., C. G. Chambliss, A. T. Adesogan, and M. B. Adjei. 2006. Silage Harvesting, Storing, and Feeding. This document is SS-AGR-177, one of a series of the Agronomy Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida. This publication is also part of the Florida Forage Handbook, an electronic publication of the Agronomy Department.

McDonald P., A.R. Henderson, and S.J.E. Heron. 1991. The Biochemistry of Silage. 2nd ed. Marlow. Chalcombe Publication. UK.

McIllroy R.J. 1977. Pengantar Budidaya Padang Rumput Tropika. Pradnya Paramita, Jakarta

Meeske, R., R. Asbell, Z.G. Weinberg, and T. Kipnis. 1993. Ensiling forage sorghum at two stages of maturity with the addition of lactic acid bacterial inoculant. J. Anim. Feed Sci. and Tech. 43:165-175.

Messersmith, T.L. 1973. Evaluation of Dried Paunch Feed as Roughages Source in Ruminant Finishing Rations. M.A. Thesis. Department of Animal Science. University of Nebraska.

Muck, R.2004. Inoculants for Corn Silage. University of Wisconsin Board of Regents. College of Agricultural and Life Sciences. University of Wisconsin. Medison.

Muck, R.E., 1987. Butyric Acid In Silage: Why It Happens. US Dairy Forage Research Center.

Nahm, K.H. 1992. Practical Guide to Feed, Foreage and Water Analysis. Yoo Han Publishing, Seoul. Korea.

Nayigihugu, V., D.W. Kellog, Z.B. Johnson, M. Scott, and K.S. Anschutz. 1995. Effect of adding levels molasses on composition of bermudagrass (Cynodon dactylon) silage. J. Anim Sci. 73 Suppl 1:200

Pope, G.R. 1973. Silage. A4-H Dairy Science Exercise. Wisconsin.

Ricci. 1997. A.method of manure disposal for beef packing operation. First Interin Tech.Rep.EPA-600/2-77-103.

Rodriguez, A.A., S.R. Rust, M.T. Yokoyama, and E.O. Requelme. 1994. Microbial inoculant and enzymes in forage sorgum ensiled under temperate and tropical environments. 1. Microbial succession. J. Anim. Sci., 72. Suppl. 1 / J. Dairy Sc., 77 Suppl. 1,p.301

Schroeder. J., W. 2004. Silage Fermentation and Preservation. Extension Dairy Specialist North Dacota State University Fargo, North Dacota. AS-1254, June 2004.

Shaver, R.D., 2008. Harvest and Storage of High-Quality Corn Silage for Dairy Cows. Department of Dairy Science. College of Agricultural and Life Sciences. University of Wisconsin – Madison. University of Wisconsin – Extension

Steel, R.G.D. and J.H. Torrie. 1991. Prinsip dan Prosedur Statistika, Suatu Pendekatan Biometrik. PT. Gramedia Pustaka Utama. Jakarta

Tedjowahjono S. 1987. Potensi Tetes sebagai Hasil Samping Pabrik Gula dan Manfaatnya. Pusat Penelitian Perkebunan Gula Indonesia, Pasuruan.

Van Saun and A. Jud Heinrichs.2008. Troubleshooting Silage Problems. Problems Problems. The Pennsylvania State University. 324 Henning Building. University Park, PA 16802.

Witherow, J.L. and S. Lammers. 1976. Pounch and Viscera Handling.Pp.37-66 In Workshop (1973) On in-Plant Waste Reduction In The Meat Industry. Compiled by J.L.Witherow On J.F.Scief.Environ. Prot.Technol.Ser.EPA-600/2-76-214.

Zimmerman, C. 2008. Silage Fermentation Analysis. © Blue Seal Feeds, Inc. – February, 2002.