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EFFECT OF MOLASSES, RICE BRAN AND TAPIOCA FLOUR AS ADDITIVES ON THE QUALITY AND DIGESTIBILITY OF CASSAVA LEAF SILAGE

Asep Sudarman*, Rizk i Nurul Amalia and Dewi Apri Astuti
Department of Nutrition and Feed Technology, Faculty of Animal Science
Bogor Agricultural University, Bogor, 16680, Indonesia
*Corresponding author: asudarman@ipb.ac.id

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ABSTRACT

Cassava (*Manihot esculenta* Crantz) leaves, the waste of the tapioca flour industry, contain high levels of crude protein that can be used as a complementary forage for low quality field grass. The study was conducted from September to November 2009 in the Dairy Nutrition Laboratory of Bogor Agricultural University. This study sought to analyze the quality of cassava leaf silages supplemented with different additives such as molasses, rice bran and tapioca flour and to determine their digestibility and fermentability *in vitro*. This study consisted of two experiments: 1) measuring the physical properties of silages and 2) measuring the *in vitro* fermentability and digestibility of silages. A completely randomized design was used in the first experiment and a randomized complete block design in the second experiment. The treatments were: K (cassava leaves without additive) as a control, M5 (K + 5% molasses), M10 (K + 10% molasses), RB5 (K + 5% rice bran), RB10 (K + 10% rice bran), T5 (K + 5% tapioca flour) and T10 (K + 10% tapioca flour). The variables measured were colour, odour, pH, water content, NH_3, VFA, and dry matter and organic matter digestibility. Data were analyzed using Analysis of Variance (ANOVA) and any significant differences between means were further tested using Least Significant Difference (LSD). The results showed that the silages with any additives had better quality than that of the control. The quality of silages with molasses additives at either a 5% or 10% level were better than those of silages with other additives. Types and levels of additives significantly affected ($P<0.05$) *in vitro* fermentability and digestibility. In overall quality assessments, a molasses additive at a level of 5% produced the best quality of silage.

Key words : crude protein, fermentability, in vitro, physical property, sheep

INTRODUCTION

Traditional farming system usually relies on feeding sheep with field grass which is low in nutrient content thus resulting in low production. This is despite the fact that there is available crop waste containing good nutrient content that can be used as animal feed such as cassava leaves. Indonesia is one of the major cassava producing countries. Indonesia's cassava production in 2013 amounted to 23,936,921 tons with the harvested area of 1,065,572 ha (Statistics Indonesia, 2014). Utilization of cassava tubers for animal feed competes with human food need including its use after processing to become tapioca flour. Cassava leaves, on the other hand, are often a waste of tapioca flour industry therefore it has a potential to be used as animal feed. Cassava leaves are high in protein content of 20.2% (Marjuki *et al*. 2008), ranging from 23.2 to 35.9% depending on the cultivars and decreasing with age (Wobeto *et al*. 2006). It is good as a complementary forage for low quality tropical grass. Cassava leaves also contain branched amino acid of isoleucine 4.5, leucine 8.2 and valine 5.6 g/100g protein (Eggum 1970). Branched chain amino acids are beneficial for development of ruminal microorganisms and improved efficiency of ruminal fermentation *in vitro*. Provision of branched chain amino acids increase the digestibility of diet (Yang 2002), the total VFA yields and the degradability of NDF (Zhang et al. 2013). However, the utilization of cassava leaves for fodder must be careful, because they contain hydrocyanic acid (HCN) causing poison to livestock.

Indonesia, because it is located in the tropics, has two seasons which may affect the availability of forage for ruminants. In the rainy season, there is an abundance of forage sources including cassava leaves, but in the dry season the availability of forage is limited. Therefore, it needs technologies for forage preservation, one of which is silage making.

The basic principle of making silage is to create acidic conditions as soon as possible that can suppress the growth of spoilage bacteria. Besides being able to extend the shelf life, silage can also increase the palatability of forage. Therefore, during the preparation of silage, substances are often added as additives in order to accelerate the acidic conditions. One type of additives that can be added is carbohydrate source feeds. Carbohydrate-rich ingredients can stimulate the development of lactic acid bacteria (LAB) on the fermentation process (McDonald *et al*. 1991; Yitbarek and Tamir 2014). The activity of LAB causes the decrease in pH value of silage. Molasses as one of ingredients containing high water soluble carbohydrate (37.5% of DM (Man and Wiktorsson, 2002)) is usually added 3-5% (Moran 2005) up to 10% (Mühlbach 2000) during silage preparation.

Since most ruminant animals are usually reared by smallholder farmer, carbohydrate sources that can be used as additive to silage should therefore be easily obtained by them. This experiment aimed to study the effect of various sources and levels of carbohydrate use as additives on the quality of cassava leaf silage and its digestibility in vitro.

MATERIALS AND METHODS

The study was conducted from September to November 2009 in the Dairy Nutrition Laboratory of Bogor Agricultural University. This study consisted of two consecutive experiments, i.e., (1) measuring the physical quality of silage made with the addition of different levels and sources of additive and (2) measuring *in vitro* digestibility and fermentability of silage made in experiment one.

Silage Quality

Fresh cassava parts (leaves, top stalks and top trunks) obtained from the cassava flour factory waste were chopped into pieces of 2-3 cm length and withered at ambient temperature until their moisture content reached around 60% which is required for making good silage. Subsequently, 500 g of cassava leaves were thoroughly mixed with either 5% or 10% of each additives. They were then put into a five kg capacity of transparent heat resistant plastic bag. Anaerobic condition was made by removing free air from the plastic bag using a vacuum pump. The bag was then tied up and put into a black vinyl plastic bag to protect silage from sunlight to minimize heating and stored at ambient temperature for 28 days. The carbohydrate source used as additives were molasses, rice bran and tapioca flour. After 28 days of fermentation, the vinyl bags were opened and the quality of silages were assessed.

This study used completely randomized design (CRD) using seven treatments with three replicates. The treatments were: C (Control) = cassava leaves without additives, M5 = C + 5% molasses, M10 = C + 10% molasses, RB5 = C + 5% rice bran, RB10 = C + 10% rice bran, T5 = C + 5% tapioca flour, and T10 = C + 10% cassava flour. The variables measured were odour, colour, pH and moisture content of the silages.

In vitro Digestibility and Fermentability Measurement

Silages obtained from the first experiment were used in this experiment. The treatments were similar with those in first experiment, i.e., C (Control) = mixture of cassava leaves, stalk and green stem without additives, M5 = C + 5% molasses, M10 = C + 10% molasses, RB5 = C + 5% rice bran, RB10 = C + 10% rice bran, T5 = C + 5% tapioca flour, and T10 = C + 10% cassava flour.

Experimental design used was Randomized Block Design (RBD) using seven treatments with three rumen liquors of sheep as replicates/groups. Rumen liquors were obtained from slaughter house directly at the time of the animals were slaughtered. Variables measured were concentrations of NH3 (ammonia) and total volatile fatty acids (VFA) as well as dry matter and organic matter digestibility. Ammonia concentration were analyzed using method of Micro Diffusion (Conway 1962) and concentration of VFA total were measured using Steam Distillation Method (General Laboratory Procedures, 1966). Dry matter and organic matter digestibility were analyzed using the methods of Tilley and Terry (1966). All the analysis were done in the Laboratory of Dairy Nutrition of Bogor Agricultural University.

Data, except for variables of colour and smell of silages in experiment one, from both experiments were analyzed using analysis of variance (ANOVA) and treatment means were separated using Least Significance Difference (LSD) (Steel and Torrie 1980). Variables of colour and smell of silages were analyzed using descriptive statistical analysis.

Assessment of Overall Quality of Silage

Overall quality of the silages was determined using the scoring method. Score for colour of silages were 1 for brown, 2 for yellowish green, and 3 for brownish green. Score for the odour of silage were 1, 2, and 3 for silage that had a foul odour, mild sour odour, and strong sour odour, respectively. Score for pH was according to Skerman and Riveros (1990) that classified good quality silage as those having a pH of < 4.2, medium quality as having a pH of 4.3 to 4.5, and poor quality silage as having pH > 4.5. The scores given for each classification were 3, 2, and 1, respectively. Scoring for the VFA, NH3, IVDMD and IVOMD were divided into three classes, i.e., low, intermediate and high which were given the scores of 2, 4, and 6, respectively.

In vitro digestibility and fermentability of silage that would be further utilized by animal body were considered to be more important than the physical characteristics of silage produced. In determining the quality of silage, therefore, score for the odour, colour and pH were weighted by a factor of one, while scoring for VFA, NH_3, IVDMD and IVOMD were weighted by a factor of two.

RESULTS AND DISCUSSION

Silage Quality

The colour of silages produced are shown in Figure 1 and Table 1 and odour of silages produced are shown in Table 1. Except for control group (C) which had a yellowish green colour, other groups had brownish green colour (Fig. 1). All silages did not show any spoilage during the ensiling process. Odour test showed that only treatments M5 and M10 having a sour (lactic acid) smell, while the other groups had mild sour smell. Good quality silage has a characteristic yellowish green up to brownish green colour (Gallaher and Pitman 2000) depending upon silage material and has pleasant, sour and sweet smell (Kaiser et al. 2004). This indicated that the M5 and M10 had best physical properties of silages. Man and Wiktorsson (2002) reported the, silage colour turned from greenish yellow, to brownish yellow, with addition of molasses from 6 to 9%. The silage colour was getting darker with higher proportion of molasses.

Table 1. Physical properties of cassava leaf silage fermented with different additives

Treatment[1]	Colour	Odour	pH	Moisture
C	Yellowish green	Mild sour	4.30 ± 0.01^{b}	73.39 ± 6.56^{d}
M5	Brownish green	Sour	3.95 ± 0.02^{a}	68.18 ± 6.67^{bc}
M10	Brownish green	Sour	3.88 ± 0.01^{a}	65.08 ± 1.05^{b}
RB5	Brownish green	Mild sour	4.22 ± 0.01^{b}	73.30 ± 4.50^{d}
RB10	Brownish green	Mild sour	4.21 ± 0.03^{b}	71.59 ± 4.50^{cd}
T5	Brownish green	Mild sour	4.29 ± 0.01^{b}	70.10 ± 4.28^{cd}
T10	Brownish green	Mild sour	4.23 ± 0.02^{b}	59.59 ± 3.77^{a}
$LSD_{0.05}$			0.130	3.932

Values in the same column with different superscripts were significantly different (P<0.05).
[1]C (Control) = mixture of cassava leaves, stalk and green stem without additives; M5 = C + 5% molasses; M10 = C + 10% molasses; RB5 = C + 5% rice bran; RB10 = C + 10% rice bran; T5 = C + 5% tapioca flour; and T10 = C + 10% cassava flour

Figure 1. Colour of silages produced after fermentation process for 30 days. C (Control) = mixture of cassava leaves, stalk and green stem without additives; M5 = C + 5% molasses; M10 = C + 10% molasses; RB5 = C + 5% rice bran; RB10 = C + 10% rice bran; T5 = C + 5% tapioca flour; and T10 = C + 10% tapioca flour.

The inclusion of additives significantly (P <0.05) decreased pH and moisture content of silage. Table 1 shows that the pH values of cassava leaf silage with additives were lower compared with that of control group without additive. The water content of silages with additives were 59.52 – 73.30% while water content of that without additives was 73.39%. This suggests that the addition of additives can improve the quality of silage, so that silage can be conserved for long time. The same result was reported that molasses addition decreased pH and increased dry matter content of cassava leaf silage (Man and Wiktorsson 2002). M10 and M5 groups had significantly lower (P<0.05) pH values (3.88 and 3.95) than those of other groups (range 4.21 – 4.30). The range of silage pH in the present study was not different with the results of Nguyen Thi Loc et al. (2000) that pH of cassava leaf silage after fermentation for 28 days have pH values ranging from 3.6 to 4.2. Moran (2005) stated that the lower the pH of the silage the lower the activity of spoilage bacteria.

The lowest moisture content, in the present study was of T10 group (59.59%) while the highest was of C group (73.39%). All the silages could be categorized into good quality silages. According to Moran (2005) good silage has moisture content ranged between 50-75%. Our results were also similar with those of Nguyen Thi Hoa Ly et al. (2000) that the cassava leaf silage fermented for 28 days has a moisture content ranging from 66.2 to 71.2%. Moisture content above 75% will reduce palatability and feed intake (Moran 2005).

In vitro Digestibility and Fermentability Measurement

Digestibility of nutrients is one important measure in determining the quality of the feed material. The addition of additives into cassava leaf silage significantly (P <0.05) increased dry matter digestibility (DMD) and organic matter digestibility (OMD). Table 2 shows that silages with the addition of any additive had DMD and OMD values higher than that of control group (without additive addition). The highest DMD and OMD values of T10 (10% tapioca flour) treatment (64.77% and 62.87%, respectively) were due to dry matter and organic matter contents of T10 was higher compared to other treatments.

Table 2. IVDMD, IVOMD, and concentration of VFAs and ammonia of cassava leaf silage fermented with different additives.

Treatment	IVDMD (%)	IVOMD (%)	VFA (mM)	NH3 (mM)
C	51.49±2.34[a]	49.13±2.06[a]	143.51±35.37[b]	16.71±2.62[b]
M5	57.17±2.74[b]	54.20±2.68[c]	161.71±33.18[cd]	13.25±2.77[a]
M10	57.18±1.28[b]	53.87±0.82[bc]	148.91±21.51[bc]	13.65±2.12[a]
RB5	52.30±1.05[a]	49.47±1.07[a]	177.17±10.34[d]	16.70±0.64[b]
RB10	51.66±2.87[a]	53.40±5.15[bc]	170.25±12.13[d]	16.27±2.75[b]
T5	55.54±1.68[b]	51.83±3.95[b]	171.71±12.00[d]	13.07±1.47[a]
T10	64.77±4.75[c]	62.87±4.07[d]	107.03±21.54[a]	12.01±0.82[a]
$LSD_{0.05}$	2.151	2.142	17.534	1.686

Values in the same column with different superscripts were significantly different (P<0.05).
[1]C (Control) = mixture of cassava leaves, stalk and green stem without additives; M5 = C + 5% molasses; M10 = C + 10% molasses; RB5 = C + 5% rice bran; RB10 = C + 10% rice bran; T5 = C + 5% tapioca flour; and T10 = C + 10% cassava flour.

The addition of rice bran, in average, caused lower DMD and OMD of silage than that of silage with the addition of molasses or tapioca flour. Since rice bran contains higher fiber than that of cassava flour and molasses (Winugroho 1999; Hermiati et al. 2011), therefore the addition of rice bran increased crude fiber content of silage. It is well known that the higher the fiber content of the feed the lower the digestibility of the feed (Migwi et al. 2013). Other possible reason of lower DMD and OMD of rice bran addition is due to the unsaturated fat content of rice bran. This was supported by the result of Lunsin et al. (2012) that the population of total ruminal bacteria was significantly lower on the rice bran oil supplemented diet. Unsaturated fat is known to be "toxic" for microbial rumen (Maia et al. 2007) causing lower number of microbes and hence reduce the ability to digest feed.

Concentration of Volatile Fatty Acids (VFA) and Ammonia (NH_3)

The results showed that the addition of additives into cassava leaf silage significantly ($P<0.05$) affected VFA concentration. With the exception of tapioca at 10% (T10), silages with the addition of additives had VFA concentrations higher than that of control group (without additive addition). VFA concentration in the rumen is used as an indicator of feed fermentability. Table 2 shows that, on average, the highest concentration of VFA was with rice bran additive followed by molasses and tapioca, i.e. 173.71, 155.31, and 139.37 mM, respectively. Mean VFA concentration of cassava leaf silage in this experiment ranged from 107.03 to 177.17 mM. This is still in normal range of the VFA concentrations required for optimal growth of rumen microbes that is 80-160 mM. Wang and Song (2001) reported that high carbohydrate level of the feed increased fermentation in the rumen as indicated by higher number of bacteria and consequently increase VFA production. Further, they reported that VFA production was also affected by sources of carbohydrates used.

For all additives, inclusion at the 10% level did not show any benefit to VFA concentration compared to the 5% level (Table 2). Even, with tapioca additives, it was seen that 10% inclusion resulted in lower ($P<0.05$) VFA concentration compared to that of 5% inclusion. This may be explained that more VFA produced in T10 has been used for more microbial growth. Rumen microbes need carbon dioxide, nitrogen, sodium, and volatile fatty acids to grow (Hungate et al. 1964). Due to the increasing population of rumen microbes in T10 resulted in the higher feed digestibility in T10 than that in T5, as it is shown in Table 2. Other possibility is that the inclusion of 10% additives did not give added benefit compared to 5% inclusion in VFA concentration was due to the addition of 10% resulted in pH of silage to be lower. This is supported by Peters et al. (1989) who reported that total production of VFA was lower at low than at high initial pH of diet.

The inclusion of additives significantly affected ($P<0.05$) the concentrations of NH_3. Table 2 shows that silages with the addition of molasses and tapioca had NH_3 lower than that of rice bran additive or that of the control (without additive). This indicated a reduction in protein degradation of molasses and tapioca additives. Molasses and tapioca contain high water soluble carbohydrate which is used by lactic acid bacteria (LAB) during ensilage process resulting in rapidly decreasing pH (Mühlbach 2000). Higher lactic acid in silage tended to decrease protein degradation by rumen microbes resulted in lower NH_3 production (Jaakkola and Huhtanen 1989).. The advantage of this is that much more protein escape from rumen to be utilized further by ruminant animal host for production purpose (Kempton et al. 1977). The same results using additive of formic acid or *Lactobacillus plantarum* during ensilaging process of alfalfa have been reported (Nagel and Broderick 1992; Contreras-Govea et al. 2013). On the other hand, due to its higher protein content, silage treated with rice bran (RB5 and RB10) had higher NH_3. The higher protein content of feed will result in higher NH_3 concentration as a result of the increasing proteolytic activity (Haaland *et al*. 1982). In addition, Orden et al. (2000) reported that rice bran supplementation affect the degradation of crude protein. The protein content of each additive were 9.85%, 4.61% and 0.51% for rice bran, molasses, and tapioca, respectively (Table 3).

Table 3. Nutrient composition of the additives

Nutrient	Molasses	Rice bran	Cassava tuber meal
Dry matter	59.8	87.7	87.9
Crude protein	4.61	9.85	0.51
Soluble carbohydrate	72.1	4.12	62.1
NDF	0.00	24.9	20.0
ADF	0.00	12.3	0.16

Source: Rusdy (2015)

High concentrations of NH_3 due to high rate of protein breakdown in the rumen is not beneficial. Ammonia produced will be used by microbes in the rumen for the formation of microbial protein and reduce the protein proportion to be used by animal host. Mean NH_3 concentration in this experiment ranged from 12.01 to 16.71 mM, which were in the optimum range of the rumen NH_3, i.e., 85 - 300 mg/l or equal to 4.99 – 17.61 mM (McDonald *et al*. 2010).

Overall quality assessment

The overall quality assessment of the silages were shown in Table 4. Based on the physical characteristics of the silage, the highest score was obtained by molasses additive, followed then by rice bran and tapioca and control group, i.e., 9, 7, 7, and 6, respectively. Based on the rumen fermentation characteristics, the highest score was achieved by molasses and tapioca, followed by rice bran and control, i.e., 17, 17, 12, and 10 respectively The silage with molasses additive gained the highest overall score, followed by the addition of tapioca flour, rice bran and control, i.e., in average 26, 24, 19, and 16, respectively. This may be due to molasses containing higher water soluble carbohydrate (Table 3) which is easily used by the microbes during ensiling.

Table 4. Overall quality of cassava leaf silage fermented with different additives based on scoring method

Treatment[1]	VFA	NH3	IVDMD	IVOMD	pH	Colour	Odour	Total
C	4	2	2	2	2	2	2	16
M5	6	4	4	4	3	3	3	27
M10	4	4	4	4	3	3	3	25
RB5	6	2	2	2	2	3	2	19
RB10	6	2	2	2	2	3	2	19
T5	6	4	4	2	2	3	2	23
T10	2	4	6	6	2	3	2	25

[1]C (Control) = mixture of cassava leaves, stalk and green stem without additives; M5 = C + 5% molasses; M10 = C + 10% molasses; RB5 = C + 5% rice bran; RB10 = C + 10% rice bran; T5 = C + 5% tapioca flour; and T10 = C + 10% cassava flour.

The same result was reported by Rusdy (2015) who found that applying additive of 5% molasses in making silage of *Chromolaena odourata* gave the best result compared to other additives (cassava tuber flour, maize meal, and rice bran). In vivo study (Sudarman et al. 2016) showed the advantages of feeding 20% cassava leaf silage with 5% molasses additive that greatly improved sheep performance similar to that achieved by feeding concentrate.

CONCLUSIONS

The total scoring method demonstrates that cassava leaf silage with molasses additives produce better quality silage than the other tested additives. The addition of 5% molasses obtained higher score than that of the addition of 10% molasses. Therefore, the addition of 5% molasses makes good quality cassava leaf silage for sheep.

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